BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images may include (but are not limited to):

- BLACK BORDERS
- TEXT CUT OFF AT TOP, BOTTOM OR SIDES
- FADED TEXT
- ILLEGIBLE TEXT
- SKEWED/SLANTED IMAGES
- COLORED PHOTOS
- BLACK OR VERY BLACK AND WHITE DARK PHOTOS
- GRAY SCALE DOCUMENTS

IMAGES ARE BEST AVAILABLE COPY.

As rescanning documents *will not* correct images, please do not report the images to the Image Problem Mailbox.

WEST

Help | Logout

Main Menu | Search Form | Posting Counts | Show S Numbers | Edit S Numbers

Search Results -

Terms	Documents
l13 and 514/1.ccls.	0

Database: US Patents Full-Text Database

Refine Search: `l13 and 514/1.ccls.`

Search History

DB Name	Query	Hit Count	Set Name
USPT	l13 and 514/1.ccls.	0	L17
USPT	l13 and 800/18.ccls.	8	L16
USPT	l13 and 800/8.ccls.	1	L15
USPT	l13 and 800/3.ccls.	2	L14
USPT	l11 or l12	221	L13
USPT	l11 and l7	49	L12
USPT	l3 or l9	221	L11
USPT	l3 or l8	2389	L10
USPT	l1 and l8	184	L9
USPT	elastase	2352	L8
USPT	l6 and l4	9543	L7
USPT	svas or hypertension or atherosclerosis	16464	L6
USPT	supravalvular adj arotic adj stenosis	0	L5
USPT	method near3 (screen$ or treat$)	117073	L4
USPT	l1 and l2	45	L3
USPT	elastin or eln	1797	L2
USPT	transgen$ near5 (mouse or mice)	2104	L1

WEST

Help | Logout

Main Menu | Search Form | Posting Counts | Show S Numbers | Edit S Numbers

Generate Collection

Search Results - Record(s) 1 through 8 of 8 returned.

☐ 1. Document ID: US 5849996 A
Entry 1 of 8 File: USPT Dec 15, 1998

US-PAT-NO: 5849996
DOCUMENT-IDENTIFIER: US 5849996 A
TITLE: BCR/ABL transgenic animals as models for Philadelphia chromosome positive chronic myelogenous and acute lymphoblastic leukemia

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Image

☐ 2. Document ID: US 5849993 A
Entry 2 of 8 File: USPT Dec 15, 1998

US-PAT-NO: 5849993
DOCUMENT-IDENTIFIER: US 5849993 A
TITLE: Transgenic mice as a model for metabolic bone diseases

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Image

☐ 3. Document ID: US 5705732 A
Entry 3 of 8 File: USPT Jan 6, 1998

US-PAT-NO: 5705732
DOCUMENT-IDENTIFIER: US 5705732 A
TITLE: Universal donor cells

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Image

☐ 4. Document ID: US 5602306 A
Entry 4 of 8 File: USPT Feb 11, 1997

US-PAT-NO: 5602306
DOCUMENT-IDENTIFIER: US 5602306 A
TITLE: Synthesis of functional human hemoglobin and other proteins in erythroid tissues of transgenic animals

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Image

☐ 5. Document ID: US 5583009 A
Entry 5 of 8 File: USPT Dec 10, 1996

US-PAT-NO: 5583009
DOCUMENT-IDENTIFIER: US 5583009 A
TITLE: Method of preparing recombinant proteins in transgenic animals containing metallothionein gene elements that bestow tissue-independent copy number-dependent, position-indepedent gene expression

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Image

☐ 6. Document ID: US 5574206 A
Entry 6 of 8 File: USPT Nov 12, 1996

US-PAT-NO: 5574206
DOCUMENT-IDENTIFIER: US 5574206 A
TITLE: Transgenic mouse carrying a non-infectious HIV genome

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Image

☐ 7. Document ID: US 5491283 A
Entry 7 of 8 File: USPT Feb 13, 1996

US-PAT-NO: 5491283
DOCUMENT-IDENTIFIER: US 5491283 A
TITLE: BRC/ABL transgenic animals as models for Philadelphia chromosome positive chronic myelogenous and acute lymphoblastic leukemia

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Image

☐ 8. Document ID: US 5221778 A
Entry 8 of 8 File: USPT Jun 22, 1993

US-PAT-NO: 5221778
DOCUMENT-IDENTIFIER: US 5221778 A
TITLE: Multiplex gene regulation

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Image

Generate Collection

Terms	Documents
113 and 800/18.ccls.	8

Display 20 Documents including document number 8

Display Format: TI Change Format

Main Menu | Search Form | Posting Counts | Show S Numbers | Edit S Numbers

Help | Logout

WEST

Help | Logout

Main Menu | Search Form | Posting Counts | Show S Numbers | Edit S Numbers

Generate Collection

Search Results - Record(s) 1 through 1 of 1 returned.

☐ 1. Document ID: US 5602301 A

Entry 1 of 1 File: USPT Feb 11, 1997

US-PAT-NO: 5602301
DOCUMENT-IDENTIFIER: US 5602301 A
TITLE: Non-human mammal having a graft and methods of delivering protein to myocardial tissue

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Image

Generate Collection

Terms	Documents
113 and 800/8.ccls.	1

Display 20 Documents including document number 1

Display Format: TI Change Format

Main Menu | Search Form | Posting Counts | Show S Numbers | Edit S Numbers

Help | Logout

WEST

Help | Logout

Main Menu | Search Form | Posting Counts | Show S Numbers | Edit S Numbers

Generate Collection

Search Results - Record(s) 1 through 2 of 2 returned.

☐ 1. Document ID: US 5837875 A

Entry 1 of 2 File: USPT Nov 17, 1998

US-PAT-NO: 5837875
DOCUMENT-IDENTIFIER: US 5837875 A
TITLE: Transgenic mouse containing an IGF-1 transgene

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Image

☐ 2. Document ID: US 5718883 A

Entry 2 of 2 File: USPT Feb 17, 1998

US-PAT-NO: 5718883
DOCUMENT-IDENTIFIER: US 5718883 A
TITLE: Transgenic animal model for autoimmune diseases

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Image

Generate Collection

Terms	Documents
113 and 800/3.ccls.	2

Display 20 Documents including document number 2

Display Format: TI Change Format

Main Menu | Search Form | Posting Counts | Show S Numbers | Edit S Numbers

Help | Logout

```
=> d his

     (FILE 'HOME' ENTERED AT 09:36:29 ON 14 JAN 2000)

     FILE 'MEDLINE, CAPLUS, BIOSIS, SCISEARCH' ENTERED AT 09:36:41 ON 14 JAN
     2000
L1         69713 S TRANSGEN?(5A)(MOUSE OR MICE)
L2         19573 S ELASTIN OR ELN
L3           108 S L1 AND L2
L4         10505 S ELASTASE(6A)INHIBIT?
L5          1061 S SUPRAVALVULAR(W)AORTIC(W)STENOSIS OR SVAS
L6        556163 S HYPERTENSION OR ATHEROSCLEROSIS
L7        557176 S L5 OR L6
L8           194 S L7 AND L4
L9             4 S L3 AND L6
L10            4 S L3 AND L4
L11           54 DUP REM L3 (54 DUPLICATES REMOVED)
L12            4 DUP REM L9 L10 (4 DUPLICATES REMOVED)

=> d bib 1-4 l12


L12  ANSWER 1 OF 4  MEDLINE                                  DUPLICATE 1
AN   1999225536     MEDLINE
DN   99225536
TI   Targeted overexpression of elafin protects mice against cardiac
     dysfunction and mortality following viral myocarditis.
AU   Zaidi S H; Hui C C; Cheah A Y; You X M; Husain M; Rabinovitch M
CS   Program in Cardiovascular Research, The Hospital for Sick Children,
     Toronto, Ontario, Canada.
SO   JOURNAL OF CLINICAL INVESTIGATION, (1999 Apr) 103 (8) 1211-9.
     Journal code: HS7. ISSN: 0021-9738.
CY   United States
DT   Journal; Article; (JOURNAL ARTICLE)
LA   English
FS   Abridged Index Medicus Journals; Priority Journals; Cancer Journals
EM   199908
EW   19990801

L12  ANSWER 2 OF 4  MEDLINE
AN   1999278197     MEDLINE
DN   99278197
TI   EVEC, a novel epidermal growth factor-like repeat-containing protein
     upregulated in embryonic and diseased adult vasculature [see comments].
CM   Comment in: Circ Res 1999 May 28;84(10):1234
AU   Kowal R C; Richardson J A; Miano J M; Olson E N
CS   Department of Internal Medicine, University of Texas Southwestern Medical
     Center, Dallas, Texas 75235-9148, USA.
SO   CIRCULATION RESEARCH, (1999 May 28) 84 (10) 1166-76.
     Journal code: DAJ. ISSN: 0009-7330.
CY   United States
DT   Journal; Article; (JOURNAL ARTICLE)
LA   English
FS   Priority Journals
OS   GENBANK-AF093118; GENBANK-AF093119
EM   199908
EW   19990804
```

L12 ANSWER 3 OF 4 SCISEARCH COPYRIGHT 2000 ISI (R)
AN 1998:920166 SCISEARCH
GA The Genuine Article (R) Number: 142NE
TI Reduced transplant arteriosclerosis in plasminogen-deficient mice
AU Moons L; Shi C W; Ploplis V; Plow E; Haber E; Collen D (Reprint); Carmeliet P
CS KATHOLIEKE UNIV LEUVEN, FLANDERS INTERUNIV INST BIOTECHNOL, CTR TRANSGENE TECHNOL & GENE THERAPY, B-3000 LEUVEN, BELGIUM (Reprint); KATHOLIEKE UNIV LEUVEN, FLANDERS INTERUNIV INST BIOTECHNOL, CTR TRANSGENE TECHNOL & GENE THERAPY, B-3000 LEUVEN, BELGIUM; HARVARD UNIV, SCH PUBL HLTH, CARDIOVASC BIOL LAB, BOSTON, MA 02115; CLEVELAND CLIN FDN, JJ JACOBS CTR THROMBOSIS & VASC BIOL, CLEVELAND, OH 44195
CYA BELGIUM; USA
SO JOURNAL OF CLINICAL INVESTIGATION, (15 NOV 1998) Vol. 102, No. 10, pp. 1788-1797.
Publisher: ROCKEFELLER UNIV PRESS, 1114 FIRST AVE, 4TH FL, NEW YORK, NY 10021.
ISSN: 0021-9738.
DT Article; Journal
FS LIFE
LA English
REC Reference Count: 33
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

L12 ANSWER 4 OF 4 SCISEARCH COPYRIGHT 2000 ISI (R)
AN 94:98482 SCISEARCH
GA The Genuine Article (R) Number: MX571
TI REGULATION OF THE ALPHA-1(I) COLLAGEN PROMOTER IN VASCULAR SMOOTH-MUSCLE CELLS - COMPARISON WITH OTHER ALPHA-1(I) COLLAGEN-PRODUCING CELLS IN TRANSGENIC ANIMALS AND CULTURED-CELLS
AU BEDALOV A; BREAULT D T; SOKOLOV B P; LICHTLER A C; BEDALOV I; CLARK S H; MACK K; KHILLAN J S; WOODY C O; KREAM B E; ROWE D W (Reprint)
CS UNIV CONNECTICUT, CTR HLTH, DEPT PEDIAT, FARMINGTON, CT, 06030 (Reprint); UNIV CONNECTICUT, CTR HLTH, DEPT PEDIAT, FARMINGTON, CT, 06032; UNIV CONNECTICUT, CTR HLTH, DEPT MED, FARMINGTON, CT, 06032; THOMAS JEFFERSON UNIV, DEPT BIOCHEM & MOLEC BIOL, PHILADELPHIA, PA, 19107; DEPT VET AFFAIRS MED CTR, NEWINGTON, CT, 06111; UNIV CONNECTICUT, DEPT ANIM SCI, STORRS, CT, 06269; UNIV ZAGREB, SCH MED, 41000 ZAGREB, CROATIA; UNIV ZAGREB, SCH DENT MED, 41000 ZAGREB, CROATIA
CYA USA; CROATIA
SO JOURNAL OF BIOLOGICAL CHEMISTRY, (18 FEB 1994) Vol. 269, No. 7, pp. 4903-4909.
ISSN: 0021-9258.
DT Article; Journal
FS LIFE
LA ENGLISH
REC Reference Count: 44
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

=> d 1-54 au ti so l11

L11 ANSWER 1 OF 54 CAPLUS COPYRIGHT 2000 ACS
IN Ensley, Burt D.
TI Cosmetic compositions containing extracellular matrix proteins
SO PCT Int. Appl., 65 pp.
CODEN: PIXXD2

L11 ANSWER 2 OF 54 SCISEARCH COPYRIGHT 2000 ISI (R)
AU Lijnen H R (Reprint); VanHoef B; Vanlinthout I; Verstreken M; Rio M C; Collen D

TI Accelerated neointima formation after vascular injury in mice with stromelysin-3 (MM[redacted]1) gene inactivation
SO ARTERIOSCLEROSIS [redacted]ROMBOSIS AND VASCULAR BIOLOGY, [redacted]C 1999) Vol. 19, No. 12, pp. 2863-2870.
Publisher: LIPPINCOTT WILLIAMS & WILKINS, 227 EAST WASHINGTON SQ, PHILADELPHIA, PA 19106.
ISSN: 1079-5642.

L11 ANSWER 3 OF 54 MEDLINE DUPLICATE 1
AU Zaidi S H; Hui C C; Cheah A Y; You X M; Husain M; Rabinovitch M
TI Targeted overexpression of elafin protects mice against cardiac dysfunction and mortality following viral myocarditis.
SO JOURNAL OF CLINICAL INVESTIGATION, (1999 Apr) 103 (8) 1211-9.
Journal code: HS7. ISSN: 0021-9738.

L11 ANSWER 4 OF 54 MEDLINE
AU Kowal R C; Richardson J A; Miano J M; Olson E N
TI EVEC, a novel epidermal growth factor-like repeat-containing protein upregulated in embryonic and diseased adult vasculature [see comments].
SO CIRCULATION RESEARCH, (1999 May 28) 84 (10) 1166-76.
Journal code: DAJ. ISSN: 0009-7330.

L11 ANSWER 5 OF 54 MEDLINE
AU Warburton D; Lee M K
TI Current concepts on lung development.
SO CURRENT OPINION IN PEDIATRICS, (1999 Jun) 11 (3) 188-92. Ref: 23
Journal code: BUT. ISSN: 1040-8703.

L11 ANSWER 6 OF 54 CAPLUS COPYRIGHT 2000 ACS DUPLICATE 2
AU Lakkakorpi, Jouni; Li, Kehua; Decker, Sylvia; Korkeela, Esa; Piddington, Ronald; Abrams, William; Bashir, Muhammad; Uitto, Jouni; Rosenbloom, Joel
TI Expression of the **elastin** promoter in novel tissue sites in **transgenic mouse** embryos
SO Connect. Tissue Res. (1999), 40(2), 155-162
CODEN: CVTRBC; ISSN: 0300-8207

L11 ANSWER 7 OF 54 MEDLINE DUPLICATE 3
AU Lemack G E; Szabo Z; Urban Z; Boyd C D; Csiszar K; Vaughan E D Jr; Felsen D
TI Altered bladder function in **transgenic mice** expressing rat **elastin**.
SO NEUROUROLOGY AND URODYNAMICS, (1999) 18 (1) 55-68.
Journal code: BRQ. ISSN: 0733-2467.

L11 ANSWER 8 OF 54 BIOSIS COPYRIGHT 2000 BIOSIS
AU Brown, D. B. (1); Kong, S. K. (1); Kwak, B. C. (1); Takeuchi, T. (1); Uitto, J. (1); Bernstein, E. F. (1); Gasparro, F. P. (1)
TI The nitroxide tempol affords protection against UVR as assayed using a **transgenic mouse** molecular model of cutaneous photoaging.
SO Photochemistry and Photobiology, (June, 1999) Vol. 69, No. SPEC. ISSUE., pp. 53S-54S.
Meeting Info.: Twenty Seventh Annual Meeting of the American Society for Photobiology Washington, D.C., USA July 10-15,1999 American Society for Photobiology
. ISSN: 0031-8655.

L11 ANSWER 9 OF 54 CAPLUS COPYRIGHT 2000 ACS
IN Bernstein, Eric
TI Use of Tempol in the prevention of photoaging
SO U.S., 5 pp.
CODEN: USXXAM

L11 ANSWER 10 OF 54 SCISEARCH COPYRIGHT 2000 ISI (R)
AU Moons L; Shi C W; Ploplis V; Plow E; Haber E; Collen D (Reprint);

Carmeliet P
TI Reduced transplan[redacted]arteriosclerosis in plasminogen-deficient mice
SO JOURNAL OF CLINIC[redacted] INVESTIGATION, (15 NOV 1998) V[redacted] 102, No. 10, pp. 1788-1797.
Publisher: ROCKEFELLER UNIV PRESS, 1114 FIRST AVE, 4TH FL, NEW YORK, NY 10021.
ISSN: 0021-9738.

L11 ANSWER 11 OF 54 MEDLINE DUPLICATE 4
AU Takeuchi T; Uitto J; Bernstein E F
TI A novel in vivo model for evaluating agents that protect against ultraviolet A-induced photoaging.
SO JOURNAL OF INVESTIGATIVE DERMATOLOGY, (1998 Apr) 110 (4) 343-7.
Journal code: IHZ. ISSN: 0022-202X.

L11 ANSWER 12 OF 54 MEDLINE DUPLICATE 5
AU Uitto J; Bernstein E F
TI Molecular mechanisms of cutaneous aging: connective tissue alterations in the dermis.
SO JOURNAL OF INVESTIGATIVE DERMATOLOGY. SYMPOSIUM PROCEEDINGS, (1998 Aug) 3 (1) 41-4. Ref: 35
Journal code: C0U. ISSN: 1087-0024.

L11 ANSWER 13 OF 54 SCISEARCH COPYRIGHT 2000 ISI (R)
AU Lindahl P; Karlsson L; Hellstrom M; GebreMedhin S; Willetts K; Heath J K; Betsholtz C (Reprint)
TI Alveogenesis failure in PDGF-A-deficient mice is coupled to lack of distal
spreading of alveolar smooth muscle cell progenitors during lung development
SO DEVELOPMENT, (OCT 1997) Vol. 124, No. 20, pp. 3943-3953.
Publisher: COMPANY OF BIOLOGISTS LTD, BIDDER BUILDING CAMBRIDGE COMMERCIAL
PARK COWLEY RD, CAMBRIDGE, CAMBS, ENGLAND CB4 4DL.
ISSN: 0950-1991.

L11 ANSWER 14 OF 54 SCISEARCH COPYRIGHT 2000 ISI (R)
AU DelMonaco M; Covello S P; Kennedy S H; Gilinger G; Litwack G; Uitto J (Reprint)
TI Identification of novel glucocorticoid-response elements in human **elastin** promoter and demonstration of nucleotide sequence specificity of the receptor binding
SO JOURNAL OF INVESTIGATIVE DERMATOLOGY, (JUN 1997) Vol. 108, No. 6, pp. 938-942.
Publisher: BLACKWELL SCIENCE INC, 350 MAIN ST, MALDEN, MA 02148.
ISSN: 0022-202X.

L11 ANSWER 15 OF 54 MEDLINE DUPLICATE 6
AU Bernstein E F; Brown D B; Takeuchi T; Kong S K; Uitto J
TI Evaluation of sunscreens with various sun protection factors in a new **transgenic mouse** model of cutaneous photoaging that measures **elastin** promoter activation.
SO JOURNAL OF THE AMERICAN ACADEMY OF DERMATOLOGY, (1997 Nov) 37 (5 Pt 1) 725-9.
Journal code: HVG. ISSN: 0190-9622.

L11 ANSWER 16 OF 54 CAPLUS COPYRIGHT 2000 ACS DUPLICATE 7
AU Uitto, Jouni; Brown, Douglas B.; Gasparro, Francis P.; Bernstein, Eric F.
TI Molecular aspects of photoaging
SO Eur. J. Dermatol. (1997), 7(3), 210-214
CODEN: EJDEE4; ISSN: 1167-1122

L11 ANSWER 17 OF 54 MEDLINE DUPLICATE 8
AU Perrin S; Foster J A
TI Developmental regulation of **elastin** gene expression.

SO CRITICAL REVIEWS IN EUKARYOTIC GENE EXPRESSION, (1997) 7 (1-2) 1-10.
Ref:
78
Journal code: BEJ. ISSN: 1045-4403.

L11 ANSWER 18 OF 54 CAPLUS COPYRIGHT 2000 ACS
IN Bernstein, Eric; Uitto, Jouni
TI An in vivo and in vitro model of cutaneous photoaging, and method for identifying compds. capable of inhibiting cutaneous photodamage
SO PCT Int. Appl., 16 pp.
CODEN: PIXXD2

L11 ANSWER 19 OF 54 BIOSIS COPYRIGHT 2000 BIOSIS
AU Takeuchi, Tsunemichi (1); Gasparro, Francis P.; Brown, Douglas B. (1); Kong, Sung K. (1); Lopresti, Nicholas (1); White, Terri (1); Chang, Patrick (1); Uitto, Jouni (1); Bernstein, Eric F. (1)
TI 8-Methoxypsoralen and ultraviolet A radiation activate the human **elastin** promoter in **transgenic mice**: In vivo and in vitro evidence for gene induction.
SO Journal of Investigative Dermatology, (1996) Vol. 106, No. 4, pp. 896.
Meeting Info.: Annual Meeting of the Society for Investigative Dermatology
Washington, D.C., USA May 1-5, 1996
ISSN: 0022-202X.

L11 ANSWER 20 OF 54 BIOSIS COPYRIGHT 2000 BIOSIS
AU Katchman, Stacy (1); Wu, May; Covello, Seana; Uitto, Jouni
TI Glucocorticosteroids upregulate human **elastin** promoter activity in the skin of **transgenic mice** in an age-independent manner.
SO Journal of Investigative Dermatology, (1996) Vol. 106, No. 4, pp. 839.
Meeting Info.: Annual Meeting of the Society for Investigative Dermatology
Washington, D.C., USA May 1-5, 1996
ISSN: 0022-202X.

L11 ANSWER 21 OF 54 BIOSIS COPYRIGHT 2000 BIOSIS
AU Kong, Sung K. (1); Brown, Douglas B.; Takeuchi, Tsunemichi; Vu, Ann; Chang, Patrick H.; Uitto, Jouni; Bernstein, Eric F.
TI A novel, rapid, and sensitive method for evaluating compounds offering protection against cutaneous photodamage.
SO Journal of Investigative Dermatology, (1996) Vol. 106, No. 4, pp. 836.
Meeting Info.: Annual Meeting of the Society for Investigative Dermatology
Washington, D.C., USA May 1-5, 1996
ISSN: 0022-202X.

L11 ANSWER 22 OF 54 SCISEARCH COPYRIGHT 2000 ISI (R)
AU TAKEUCHI T (Reprint); GASPARRO F P; BROWN D B; KONG S K; LOPRESTI N; WHITE T; CHANG P; UITTO J; BERNSTEIN E F
TI 8-METHOXYPSORALEN AND ULTRAVIOLET A RADIATION ACTIVATE THE HUMAN **ELASTIN** PROMOTER IN **TRANSGENIC MICE** - IN-VIVO AND IN-VITRO EVIDENCE FOR GENE INDUCTION
SO JOURNAL OF INVESTIGATIVE DERMATOLOGY, (APR 1996) Vol. 106, No. 4, pp. 543.
ISSN: 0022-202X.

L11 ANSWER 23 OF 54 MEDLINE DUPLICATE 9
AU Bernstein E F; Gasparro F P; Brown D B; Takeuchi T; Kong S K; Uitto J
TI 8-methoxypsoralen and ultraviolet a radiation activate the human **elastin** promoter in **transgenic mice**: in vivo and in vitro evidence for gene induction.
SO PHOTOCHEMISTRY AND PHOTOBIOLOGY, (1996 Aug) 64 (2) 369-74.
Journal code: P69. ISSN: 0031-8655.

L11 ANSWER 24 OF 54 MEDLINE
AU Burn J; Goodship J
TI Developmental genetics of the heart.
SO CURRENT OPINION IN GENETICS AND DEVELOPMENT, (1996 Jun) 6 (3) 322-5.
Ref:
34
Journal code: BJC. ISSN: 0959-437X.

L11 ANSWER 25 OF 54 BIOSIS COPYRIGHT 2000 BIOSIS DUPLICATE 10
AU Bernstein, E. F.; Brown, D. B.; Kong, S. K.; Takeuchi, T.; Zhang, X.; Tan,
E. M. L.
TI Human **elastin** promoter activity is up-regulated in multiple organs by UVB radiation administered to **transgenic mice**
.
SO Journal of Investigative Medicine, (1996) Vol. 44, No. 3, pp. 226A.
Meeting Info.: Annual Meeting of the Association of American Physicians, the American Society for Clinical Investigation, and the American Federation for Clinical Research: Biomedicine '96, Medical Research from Bench to Bedside Washington, D.C., USA May 3-6, 1996
ISSN: 1081-5589.

L11 ANSWER 26 OF 54 SCISEARCH COPYRIGHT 2000 ISI (R)
AU KATCHMAN S (Reprint); WU M; COVELLO S; UITTO J
TI GLUCOCORTICOSTEROIDS UP-REGULATE HUMAN **ELASTIN** PROMOTER ACTIVITY IN THE SKIN OF **TRANSGENIC MICE** IN AN AGE-INDEPENDENT MANNER
SO JOURNAL OF INVESTIGATIVE DERMATOLOGY, (APR 1996) Vol. 106, No. 4, pp. 204.
ISSN: 0022-202X.

L11 ANSWER 27 OF 54 BIOSIS COPYRIGHT 2000 BIOSIS
AU Uitto, Jouni (1); Hsu-Wong, Sylvia; Katchman, Stacy D.; Bashir, Muhammad M.; Rosenbloom, Joel
TI Skin elastic fibres: Regulation of human **elastin** promoter activity in **transgenic mice**.
SO Chadwick, D. J. [Editor]; Goode, J. A. [Editor]. Ciba Foundation Symposium, (1995) No. 192, pp. 237-253. Ciba Foundation Symposium; The molecular biology and pathology of elastic tissues.
Publisher: John Wiley and Sons Ltd. Baffin Lane, Chichester PO 19 1UD, England.
Meeting Info.: Symposium Nairobi, Kenya November 1-3, 1994
ISSN: 0300-5208. ISBN: 0-471-95718-6.

L11 ANSWER 28 OF 54 BIOSIS COPYRIGHT 2000 BIOSIS
AU Sechler, Jan L. (1); Sandberg, Lawrence B.; Roos, Philip J.; Snyder, Ida; Amenta, Peter S.; Riley, David J.; Boyd, Charles D.
TI **Elastin** gene mutations in **transgenic mice**.
SO Chadwick, D. J. [Editor]; Goode, J. A. [Editor]. Ciba Foundation Symposium, (1995) No. 192, pp. 148-165. Ciba Foundation Symposium; The molecular biology and pathology of elastic tissues.
Publisher: John Wiley and Sons Ltd. Baffin Lane, Chichester PO 19 1UD, England.
Meeting Info.: Symposium Nairobi, Kenya November 1-3, 1994
ISSN: 0300-5208. ISBN: 0-471-95718-6.

L11 ANSWER 29 OF 54 MEDLINE DUPLICATE 11
AU Katchman S D; Del Monaco M; Wu M; Brown D; Hsu-Wong S; Uitto J
TI A **transgenic mouse** model provides a novel biological assay of topical glucocorticosteroid potency.
SO ARCHIVES OF DERMATOLOGY, (1995 Nov) 131 (11) 1274-8.
Journal code: 6WU. ISSN: 0003-987X.

L11 ANSWER 30 OF 54 BIOSIS COPYRIGHT 2000 BIOSIS DUPLICATE 12

AU Brown, Douglas B. (1); Bernstein, Eric F.; Del Monaco, Magaly (1); Katchman, Stacy (1); Wu, May (1); Urbach, Frederick; Forbes, Donald; Uitto, Jouni (1)
TI Ultraviolet irradiation activates the human **elastin** promoter in **transgenic mice**: An in vivo and in vitro model of cutaneous photoaging.
SO Journal of Investigative Dermatology, (1995) Vol. 104, No. 4, pp. 599. Meeting Info.: Annual Meeting of the Society for Investigative Dermatology
Chicago, Illinois, USA May 24-28, 1995
ISSN: 0022-202X.

L11 ANSWER 31 OF 54 BIOSIS COPYRIGHT 2000 BIOSIS DUPLICATE 13
AU Del Monaco, Magaly; Wu, May; Katchman, Stacy; Tan, Elaine M. L.; Uitto, Jouni
TI Basic fibroblast growth factor regulates human **elastin** promoter activity in **transgenic mice**.
SO Journal of Investigative Dermatology, (1995) Vol. 104, No. 4, pp. 580. Meeting Info.: Annual Meeting of the Society for Investigative Dermatology
Chicago, Illinois, USA May 24-28, 1995
ISSN: 0022-202X.

L11 ANSWER 32 OF 54 MEDLINE DUPLICATE 14
AU Bernstein E F; Brown D B; Urbach F; Forbes D; Del Monaco M; Wu M; Katchman S D; Uitto J
TI Ultraviolet radiation activates the human **elastin** promoter in **transgenic mice**: a novel in vivo and in vitro model of cutaneous photoaging.
SO JOURNAL OF INVESTIGATIVE DERMATOLOGY, (1995 Aug) 105 (2) 269-73.
Journal code: IHZ. ISSN: 0022-202X.

L11 ANSWER 33 OF 54 MEDLINE DUPLICATE 15
AU Uitto J; Hsu-Wong S; Katchman S D; Bashir M M; Rosenbloom J
TI Skin elastic fibres: regulation of human **elastin** promoter activity in **transgenic mice**.
SO CIBA FOUNDATION SYMPOSIUM, (1995) 192 237-53; discussion 253-8. Ref: 49
Journal code: D7X. ISSN: 0300-5208.

L11 ANSWER 34 OF 54 MEDLINE DUPLICATE 16
AU Westermarck J; Ilvonen E; Uitto J; Kahari V M
TI Suppression of **elastin** gene expression in dermal fibroblasts by protein phosphatase inhibitor okadaic acid.
SO BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS, (1995 Apr 6) 209 (1) 175-81.
Journal code: 9Y8. ISSN: 0006-291X.

L11 ANSWER 35 OF 54 MEDLINE DUPLICATE 17
AU Sechler J L; Sandberg L B; Roos P J; Snyder I; Amenta P S; Riley D J; Boyd C D
TI **Elastin** gene mutations in **transgenic mice**.
SO CIBA FOUNDATION SYMPOSIUM, (1995) 192 148-65; discussion 165-71.
Journal code: D7X. ISSN: 0300-5208.

L11 ANSWER 36 OF 54 CAPLUS COPYRIGHT 2000 ACS
AU Sechler, Jan Louise
TI **Elastin** gene mutations in **transgenic mice**
SO (1994) 204 pp. Avail.: Univ. Microfilms Int., Order No. DA9511991
From: Diss. Abstr. Int. B 1995, 55(12) 5209

L11 ANSWER 37 OF 54 MEDLINE DUPLICATE 18
AU Hsu-Wong S; Katchman S D; Ledo I; Wu M; Khillan J; Bashir M M; Rosenbloom J; Uitto J

TI Tissue-specific and developmentally regulated expression of human **elastin** promoter [redacted]ivity in **transgenic mice**.
SO JOURNAL OF BIOLOGICAL CHEMISTRY, (1994 Jul 8) 269 [redacted]) 18072-5.
Journal code: HIV. ISSN: 0021-9258.

L11 ANSWER 38 OF 54 SCISEARCH COPYRIGHT 2000 ISI (R)
AU BEDALOV A; BREAULT D T; SOKOLOV B P; LICHTLER A C; BEDALOV I; CLARK S H; MACK K; KHILLAN J S; WOODY C O; KREAM B E; ROWE D W (Reprint)
TI REGULATION OF THE ALPHA-1(I) COLLAGEN PROMOTER IN VASCULAR SMOOTH-MUSCLE CELLS - COMPARISON WITH OTHER ALPHA-1(I) COLLAGEN-PRODUCING CELLS IN TRANSGENIC ANIMALS AND CULTURED-CELLS
SO JOURNAL OF BIOLOGICAL CHEMISTRY, (18 FEB 1994) Vol. 269, No. 7, pp. 4903-4909.
ISSN: 0021-9258.

L11 ANSWER 39 OF 54 MEDLINE
AU Korfhagen T R; Swantz R J; Wert S E; McCarty J M; Kerlakian C B; Glasser S
W; Whitsett J A
TI Respiratory epithelial cell expression of human transforming growth factor-alpha induces lung fibrosis in **transgenic mice**.
SO JOURNAL OF CLINICAL INVESTIGATION, (1994 Apr) 93 (4) 1691-9.
Journal code: HS7. ISSN: 0021-9738.

L11 ANSWER 40 OF 54 BIOSIS COPYRIGHT 2000 BIOSIS DUPLICATE 19
AU Katchman, Stacy; Brown, Doug; Wu, May; Hsu-Wong, Sylvia; Uitto, Jouni
TI 1,25-Dihydroxyvitamin D-3 upregulates human **elastin** promoter activity in the skin of **transgenic mice**.
SO Journal of Investigative Dermatology, (1994) Vol. 102, No. 4, pp. 645.
Meeting Info.: Annual Meeting of the Society for Investigative Dermatology
Baltimore, Maryland, USA April 27-30, 1994
ISSN: 0022-202X.

L11 ANSWER 41 OF 54 MEDLINE DUPLICATE 20
AU Ledo I; Wu M; Katchman S; Brown D; Kennedy S; Hsu-Wong S; Uitto J
TI Glucocorticosteroids up-regulate human **elastin** gene promoter activity in **transgenic mice**.
SO JOURNAL OF INVESTIGATIVE DERMATOLOGY, (1994 Nov) 103 (5) 632-6.
Journal code: IHZ. ISSN: 0022-202X.

L11 ANSWER 42 OF 54 BIOSIS COPYRIGHT 2000 BIOSIS DUPLICATE 21
AU Hsu-Wong, Sylvia; Katchman, Stacy; Ledo, Isabel; Wu, May; Brown, Doug; Kennedy, Susan; Uitto, Jouni
TI Upregulation of human **elastin** promoter activity by gluco-corticosteroids in **transgenic mice**.
SO Journal of Investigative Dermatology, (1994) Vol. 102, No. 4, pp. 607.
Meeting Info.: Annual Meeting of the Society for Investigative Dermatology
Baltimore, Maryland, USA April 27-30, 1994
ISSN: 0022-202X.

L11 ANSWER 43 OF 54 MEDLINE DUPLICATE 22
AU Katchman S D; Hsu-Wong S; Ledo I; Wu M; Uitto J
TI Transforming growth factor-beta up-regulates human **elastin** promoter activity in **transgenic mice**.
SO BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS, (1994 Aug 30) 203 (1)
485-90.
Journal code: 9Y8. ISSN: 0006-291X.

L11 ANSWER 44 OF 54 MEDLINE DUPLICATE 23
AU Reitamo S; Remitz A; Tamai K; Ledo I; Uitto J
TI Interleukin 10 up-regulates **elastin** gene expression in vivo and in vitro at the transcriptional level.

SO BIOCHEMICAL JOURNAL, (1994 Sep 1) 302 (Pt 2) 331-3.
Journal code: 9YO[redacted]SSN: 0264-6021.

L11 ANSWER 45 OF 54 BIOSIS COPYRIGHT 2000 BIOSIS
AU Sechler, Jan L.; Boyd, Charles D.
TI Aortic disease in **transgenic mice** containing **elastin** gene mutations.
SO Journal of Vascular Surgery, (1994) Vol. 20, No. 1, pp. 155-156.
ISSN: 0741-5214.

L11 ANSWER 46 OF 54 MEDLINE DUPLICATE 24
AU Mauviel A; Chen Y Q; Kahari V M; Ledo I; Wu M; Rudnicka L; Uitto J
TI Human recombinant interleukin-1 beta up-regulates **elastin** gene expression in dermal fibroblasts. Evidence for transcriptional regulation in vitro and in vivo.
SO JOURNAL OF BIOLOGICAL CHEMISTRY, (1993 Mar 25) 268 (9) 6520-4.
Journal code: HIV. ISSN: 0021-9258.

L11 ANSWER 47 OF 54 BIOSIS COPYRIGHT 2000 BIOSIS
AU Ledo, Isabel; Mauviel, Alain; Wu, May; Hsu-Wong, Sylvia; Uitto, Jouni
TI Use of a **transgenic mouse** model to study the regulation of the human **elastin** promoter activity in vivo.
SO Journal of Investigative Dermatology, (1993) Vol. 100, No. 4, pp. 510.
Meeting Info.: Annual Meeting of the Society for Investigative Dermatology
Washington, D.C., USA April 28-May 1, 1993
ISSN: 0022-202X.

L11 ANSWER 48 OF 54 SCISEARCH COPYRIGHT 2000 ISI (R)
AU LEDO I (Reprint); MAUVIEL A; WU M; HSUWONG S; UITTO J
TI USE OF A **TRANSGENIC MOUSE** MODEL TO STUDY THE REGULATION OF THE HUMAN **ELASTIN** PROMOTER ACTIVITY INVIVO
SO JOURNAL OF INVESTIGATIVE DERMATOLOGY, (APR 1993) Vol. 100, No. 4, pp. 510.
ISSN: 0022-202X.

L11 ANSWER 49 OF 54 SCISEARCH COPYRIGHT 2000 ISI (R)
AU LEDO I (Reprint); MAUVIEL A; WU M; HSUWONG S; UITTO J
TI USE OF A **TRANSGENIC MOUSE** MODEL TO STUDY THE REGULATION OF THE HUMAN **ELASTIN** PROMOTER ACTIVITY INVIVO
SO CLINICAL RESEARCH, (APR 1993) Vol. 41, No. 2, pp. A441.
ISSN: 0009-9279.

L11 ANSWER 50 OF 54 CAPLUS COPYRIGHT 2000 ACS
AU Uitto, Jouni; Mauviel, Alain; Kahari, Veli-Matti
TI Cytokine modulation of collagen and **elastin** gene expression
SO Pharmacol. Skin (1993), 5, 128-38
CODEN: PHSKEY; ISSN: 1011-291X

L11 ANSWER 51 OF 54 BIOSIS COPYRIGHT 2000 BIOSIS
AU Ledo, Isabel (1); Hsu-Wong, Sylvia; Wu, May; Arita, Machiko; Bashir, Muhammad M.; Rosenbloom, Joel; Khillan, Jaspal; Uitto, Jouni
TI Expression of a human **elastin** promoter-reporter gene construct in **transgenic mice** and its up-regulation by TGF-beta-1.
SO Matrix, (1993) Vol. 13, No. 1, pp. 68-69.
Meeting Info.: Fourth International Conference on the Molecular Biology and Pathology of Matrix June 10-13, 1992
ISSN: 0934-8832.

L11 ANSWER 52 OF 54 MEDLINE
AU D'Armiento J; Dalal S S; Okada Y; Berg R A; Chada K
TI Collagenase expression in the lungs of **transgenic mice** causes pulmonary emphysema.
SO CELL, (1992 Dec 11) 71 (6) 955-61.

Journal code: CQ4. ISSN: 0092-8674.

L11 ANSWER 53 OF 54 BIOSIS COPYRIGHT 2000 BIOSIS PLICATE 25
AU HSU-WONG S; LEDO I; WU M; ARITA M; BASHIR M M; ROSENBLOOM J; KHILLAN J; UITTO J
TI EXPRESSION OF A HUMAN **ELASTIN** PROMOTER-REPORTER GENE CONSTRUCT IN **TRANSGENIC MICE**.
SO 1992 ANNUAL MEETING OF THE SOCIETY FOR INVESTIGATIVE DERMATOLOGY, BALTIMORE, MARYLAND, USA, APRIL 29-MAY 2, 1992. J INVEST DERMATOL. (1992) 98 (4), 576.
CODEN: JIDEAE. ISSN: 0022-202X.

L11 ANSWER 54 OF 54 BIOSIS COPYRIGHT 2000 BIOSIS DUPLICATE 26
AU HSU-WONG S; LEDO I; WU M; ARITA M; BASHIR M M; ROSENBLOOM J; KHILLAN J; UITTO J
TI EXPRESSION OF A HUMAN **ELASTIN** PROMOTER-REPORTER GENE CONSTRUCT IN **TRANSGENIC MICE**.
SO THIRTY-SECOND ANNUAL MEETING OF THE AMERICAN SOCIETY FOR CLINICAL NUTRITION, BALTIMORE, MARYLAND, USA, APRIL 30-MAY 2, 1992. CLIN RES. (1992) 40 (2), 188A.
CODEN: CLREAS. ISSN: 0009-9279.

```
=> d his

     (FILE 'HOME' ENTERED AT 09:36:29 ON 14 JAN 2000)

     FILE 'MEDLINE, CAPLUS, BIOSIS, SCISEARCH' ENTERED AT 09:36:41 ON 14 JAN
     2000
L1         69713 S TRANSGEN?(5A)(MOUSE OR MICE)
L2         19573 S ELASTIN OR ELN
L3           108 S L1 AND L2
L4         10505 S ELASTASE(6A)INHIBIT?
L5          1061 S SUPRAVALVULAR(W)AORTIC(W)STENOSIS OR SVAS
L6        556163 S HYPERTENSION OR ATHEROSCLEROSIS
L7        557176 S L5 OR L6
L8           194 S L7 AND L4
L9             4 S L3 AND L6
L10            4 S L3 AND L4
L11           54 DUP REM L3 (54 DUPLICATES REMOVED)
L12            4 DUP REM L9 L10 (4 DUPLICATES REMOVED)
L13       660157 S MUTATION OR KNOCKOUT

=> s l3 and l13

L14           10 L3 AND L13

=> dup rem l14

PROCESSING COMPLETED FOR L14
L15            8 DUP REM L14 (2 DUPLICATES REMOVED)

=> d 1-8 bib l15


L15  ANSWER 1 OF 8  MEDLINE
AN   1999278711     MEDLINE
DN   99278711
TI   Current concepts on lung development.
AU   Warburton D; Lee M K
CS   Childrens Hospital Los Angeles Research Institute, CA 90027, USA..
     dwarburton@chlais.usc.edu
SO   CURRENT OPINION IN PEDIATRICS, (1999 Jun) 11 (3) 188-92.  Ref: 23
     Journal code: BUT. ISSN: 1040-8703.
CY   United States
DT   Journal; Article; (JOURNAL ARTICLE)
     General Review; (REVIEW)
     (REVIEW LITERATURE)
LA   English
FS   Priority Journals
EM   199909
EW   19990904

L15  ANSWER 2 OF 8  MEDLINE
AN   1999188513     MEDLINE
DN   99188513
TI   Altered bladder function in transgenic mice expressing
     rat elastin.
AU   Lemack G E; Szabo Z; Urban Z; Boyd C D; Csiszar K; Vaughan E D Jr; Felsen
     D
```

CS James Buchanan Brady Foundation, Department of Urology, Weill Medical College of Cornell University, New York, New York 10021, USA.
SO NEUROUROLOGY AND URODYNAMICS, (1999) 18 (1) 55-68.
Journal code: BRQ. ISSN: 0733-2467.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199907
EW 19990705

L15 ANSWER 3 OF 8 SCISEARCH COPYRIGHT 2000 ISI (R)
AN 97:435847 SCISEARCH
GA The Genuine Article (R) Number: XB836
TI Identification of novel glucocorticoid-response elements in human **elastin** promoter and demonstration of nucleotide sequence specificity of the receptor binding
AU DelMonaco M; Covello S P; Kennedy S H; Gilinger G; Litwack G; Uitto J (Reprint)
CS THOMAS JEFFERSON UNIV, JEFFERSON MED COLL, DEPT DERMATOL & CUTANEOUS BIOL, 233 S 10TH ST, PHILADELPHIA, PA 19107 (Reprint); THOMAS JEFFERSON UNIV, JEFFERSON MED COLL, DEPT DERMATOL & CUTANEOUS BIOL, PHILADELPHIA, PA 19107; THOMAS JEFFERSON UNIV, JEFFERSON MED COLL, DEPT MOL PHARMACOL & BIOCHEM, PHILADELPHIA, PA 19107; THOMAS JEFFERSON UNIV, JEFFERSON INST MOL MED, PHILADELPHIA, PA 19107
CYA USA
SO JOURNAL OF INVESTIGATIVE DERMATOLOGY, (JUN 1997) Vol. 108, No. 6, pp. 938-942.
Publisher: BLACKWELL SCIENCE INC, 350 MAIN ST, MALDEN, MA 02148.
ISSN: 0022-202X.
DT Article; Journal
FS LIFE; CLIN
LA English
REC Reference Count: 30
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

L15 ANSWER 4 OF 8 MEDLINE
AN 96385451 MEDLINE
DN 96385451
TI Developmental genetics of the heart.
AU Burn J; Goodship J
CS Department of Human Genetics, University of Newcastle upon Tyne, UK. john.burn@ncl.ac.uk
SO CURRENT OPINION IN GENETICS AND DEVELOPMENT, (1996 Jun) 6 (3) 322-5.
Ref: 34
Journal code: BJC. ISSN: 0959-437X.
CY ENGLAND: United Kingdom
DT Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
(REVIEW, TUTORIAL)
LA English
FS Priority Journals
EM 199702
EW 19970204

L15 ANSWER 5 OF 8 BIOSIS COPYRIGHT 2000 BIOSIS
AN 1996:47684 BIOSIS
DN PREV199698619819
TI **Elastin** gene **mutations** in **transgenic mice.**
AU Sechler, Jan L. (1); Sandberg, Lawrence B.; Roos, Philip J.; Snyder, Ida;

Amenta, Peter S.; Riley, David J.; Boyd, Charles D.
CS (1) Dep. Mol. Bio[redacted] Princeton Univ., Princeton, N[redacted]08544 USA
SO Chadwick, D. J. [Editor]; Goode, J. A. [Editor]. C[redacted] Foundation Symposium, (1995) No. 192, pp. 148-165. Ciba Foundation Symposium; The molecular biology and pathology of elastic tissues.
Publisher: John Wiley and Sons Ltd. Baffin Lane, Chichester PO 19 1UD, England.
Meeting Info.: Symposium Nairobi, Kenya November 1-3, 1994
ISSN: 0300-5208. ISBN: 0-471-95718-6.
DT Book; Conference
LA English

L15 ANSWER 6 OF 8 MEDLINE DUPLICATE 1
AN 96159453 MEDLINE
DN 96159453
TI **Elastin** gene **mutations** in **transgenic mice.**
AU Sechler J L; Sandberg L B; Roos P J; Snyder I; Amenta P S; Riley D J; Boyd C D
CS Department of Surgery, UMDNJ-Robert Wood Johnson Medical School, New Brunswick 08903, USA.
NC HL 37438 (NHLBI)
HL 42798 (NHLBI)
HL 39869 (NHLBI)
SO CIBA FOUNDATION SYMPOSIUM, (1995) 192 148-65; discussion 165-71.
Journal code: D7X. ISSN: 0300-5208.
CY Netherlands
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199605

L15 ANSWER 7 OF 8 CAPLUS COPYRIGHT 2000 ACS
AN 1995:668015 CAPLUS
DN 123:48857
TI **Elastin** gene **mutations** in **transgenic mice**
AU Sechler, Jan Louise
CS Rutgers the State U. of N.J. and U.M.D.N.J., New Brunswick, NJ, USA
SO (1994) 204 pp. Avail.: Univ. Microfilms Int., Order No. DA9511991
From: Diss. Abstr. Int. B 1995, 55(12) 5209
DT Dissertation
LA English

L15 ANSWER 8 OF 8 BIOSIS COPYRIGHT 2000 BIOSIS
AN 1994:401736 BIOSIS
DN PREV199497414736
TI Aortic disease in **transgenic mice** containing **elastin** gene **mutations**.
AU Sechler, Jan L.; Boyd, Charles D.
CS AMDNJ-Robert Wood Johnson Med. Sch., New Brunswick, NJ USA
SO Journal of Vascular Surgery, (1994) Vol. 20, No. 1, pp. 155-156.
ISSN: 0741-5214.
DT Article
LA English

STIC-ILL

1633

From: Chen, Shin-Lin
Sent: Friday, January 14, 2000 10:34 AM
To: STIC-ILL
Subject: articles

Please provide the following articles by 1-19-2000. Thanks!
Serial No. 09/258,217.

L15 ANSWER 6 OF 8 MEDLINE DUPLICATE 1
AN 96159453 MEDLINE
DN 96159453
TI ***Elastin*** gene ***mutations*** in ***transgenic*** ***mice*** .
AU Sechler J L; Sandberg L B; Roos P J; Snyder I; Amenta P S; Riley D J; Boyd C D
CS Department of Surgery, UMDNJ-Robert Wood Johnson Medical School, New Brunswick 08903, USA.
NC HL 37438 (NHLBI)
HL 42798 (NHLBI)
HL 39869 (NHLBI)
SO CIBA FOUNDATION SYMPOSIUM, (1995) 192 148-65; discussion 165-71.
Journal code: D7X. ISSN: 0300-5208.
CY Netherlands
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199605

L15 ANSWER 7 OF 8 CAPLUS COPYRIGHT 2000 ACS
AN 1995:668015 CAPLUS
DN 123:48857
TI ***Elastin*** gene ***mutations*** in ***transgenic*** ***mice***
AU Sechler, Jan Louise
CS Rutgers the State U. of N.J. and U.M.D.N.J., New Brunswick, NJ, USA
SO (1994) 204 pp. Avail.: Univ. Microfilms Int., Order No. DA9511991
From: Diss. Abstr. Int. B 1995, 55(12) 5209
DT Dissertation
LA English

L15 ANSWER 8 OF 8 BIOSIS COPYRIGHT 2000 BIOSIS
AN 1994:401736 BIOSIS
DN PREV199497414736
TI Aortic disease in ***transgenic*** ***mice*** containing ***elastin*** gene ***mutations*** .
AU Sechler, Jan L.; Boyd, Charles D.
CS AMDNJ-Robert Wood Johnson Med. Sch., New Brunwsick, NJ USA
SO Journal of Vascular Surgery, (1994) Vol. 20, No. 1, pp. 155-156.
ISSN: 0741-5214.
DT Article
LA English

L12 ANSWER 3 OF 4 SCISEARCH COPYRIGHT 2000 ISI (R)
AU Moons L; Shi C W; Ploplis V; Plow E; Haber E; Collen D (Reprint); Carmeliet P
TI Reduced transplant arteriosclerosis in plasminogen-deficient mice

MIC next p. or RBI.55

SO JOURNAL OF CLINICAL INVESTIGATION, (15 NOV 1998) Vol. 102, No. 10, pp. 1788-1797.
Publisher: ROCKEFELLER UNIV PRESS, 1114 FIRST AVE, 4TH FL, NEW YORK, NY 10021.
ISSN: 0021-9738.

L11 ANSWER 22 OF 54 SCISEARCH COPYRIGHT 2000 ISI (R)
AU TAKEUCHI T (Reprint); GASPARRO F P; BROWN D B; KONG S K; LOPRESTI N; WHITE T; CHANG P; UITTO J; BERNSTEIN E F
TI 8-METHOXYPSORALEN AND ULTRAVIOLET A RADIATION ACTIVATE THE HUMAN ***ELASTIN*** PROMOTER IN ***TRANSGENIC*** ***MICE*** - IN-VIVO AND IN-VITRO EVIDENCE FOR GENE INDUCTION
SO JOURNAL OF INVESTIGATIVE DERMATOLOGY, (APR 1996) Vol. 106, No. 4, pp. 543.
ISSN: 0022-202X.

L11 ANSWER 26 OF 54 SCISEARCH COPYRIGHT 2000 ISI (R)
AU KATCHMAN S (Reprint); WU M; COVELLO S; UITTO J
TI GLUCOCORTICOSTEROIDS UP-REGULATE HUMAN ***ELASTIN*** PROMOTER ACTIVITY IN THE SKIN OF ***TRANSGENIC*** ***MICE*** IN AN AGE-INDEPENDENT MANNER
SO JOURNAL OF INVESTIGATIVE DERMATOLOGY, (APR 1996) Vol. 106, No. 4, pp. 204.
ISSN: 0022-202X.

L11 ANSWER 29 OF 54 MEDLINE DUPLICATE 11
AU Katchman S D; Del Monaco M; Wu M; Brown D; Hsu-Wong S; Uitto J
TI A ***transgenic*** ***mouse*** model provides a novel biological assay of topical glucocorticosteroid potency.
SO ARCHIVES OF DERMATOLOGY, (1995 Nov) 131 (11) 1274-8.
Journal code: 6WU. ISSN: 0003-987X.

L11 ANSWER 31 OF 54 BIOSIS COPYRIGHT 2000 BIOSIS DUPLICATE 13
AU Del Monaco, Magaly; Wu, May; Katchman, Stacy; Tan, Elaine M. L.; Uitto, Jouni
TI Basic fibroblast growth factor regulates human ***elastin*** promoter activity in ***transgenic*** ***mice*** .
SO Journal of Investigative Dermatology, (1995) Vol. 104, No. 4, pp. 580.
Meeting Info.: Annual Meeting of the Society for Investigative Dermatology Chicago, Illinois, USA May 24-28, 1995
ISSN: 0022-202X.

L11 ANSWER 44 OF 54 MEDLINE DUPLICATE 23
AU Reitamo S; Remitz A; Tamai K; Ledo I; Uitto J
TI Interleukin 10 up-regulates ***elastin*** gene expression in vivo and in vitro at the transcriptional level.
SO BIOCHEMICAL JOURNAL, (1994 Sep 1) 302 (Pt 2) 331-3.
Journal code: 9YO. ISSN: 0264-6021.

L11 ANSWER 47 OF 54 BIOSIS COPYRIGHT 2000 BIOSIS
AU Ledo, Isabel; Mauviel, Alain; Wu, May; Hsu-Wong, Sylvia; Uitto, Jouni
TI Use of a ***transgenic*** ***mouse*** model to study the regulation of the human ***elastin*** promoter activity in vivo.
SO Journal of Investigative Dermatology, (1993) Vol. 100, No. 4, pp. 510.
Meeting Info.: Annual Meeting of the Society for Investigative Dermatology Washington, D.C., USA April 28-May 1, 1993
ISSN: 0022-202X.

Reduced Transplant Arteriosclerosis in Plasminogen-deficient Mice

Lieve Moons,* Chengwei Shi,‡ Victoria Pioplis,§ Edward Plow,§ Edgar Haber,‡† Désiré Collen,* and Peter Carmeliet*

**Center for Transgene Technology and Gene Therapy, Flanders Interuniversity Institute for Biotechnology, B-3000 Leuven, Belgium; ‡Cardiovascular Biology Laboratory, Harvard School of Public Health, Boston, Massachusetts 02115; and §J.J. Jacobs Center for Thrombosis and Vascular Biology, the Cleveland Clinic Foundation, Cleveland, Ohio 44195*

Abstract

Recent gene targeting studies indicate that the plasminogen system is implicated in cell migration and matrix degradation during arterial neointima formation and atherosclerotic aneurysm formation. This study examined whether plasmin proteolysis is involved in accelerated posttransplant arteriosclerosis (graft arterial disease). Donor carotid arteries from wild-type B10.A2R mice were transplanted into either plasminogen wild-type ($Plg^{+/+}$) or homozygous plasminogen-deficient ($Plg^{-/-}$) recipient mice with a genetic background of 75% C57BL/6 and 25% 129. Within 15 d after allograft transplantation, leukocytes and macrophages infiltrated the graft intima in $Plg^{+/+}$ and $Plg^{-/-}$ recipient mice to a similar extent. In $Plg^{+/+}$ recipients, the elastic laminae in the transplant media exhibited breaks through which macrophages infiltrated before smooth muscle cell proliferation, whereas in $Plg^{-/-}$ recipients, macrophages failed to infiltrate the transplant media which remained structurally more intact. After 45 d of transplantation, a multilayered smooth muscle cell–rich transplant neointima developed in $Plg^{+/+}$ hosts, in contrast to $Plg^{-/-}$ recipients, in which the transplants contained a smaller intima, predominantly consisting of leukocytes, macrophages, and thrombus. Media necrosis, fragmentation of the elastic laminae, and adventitial remodeling were more pronounced in $Plg^{+/+}$ than in $Plg^{-/-}$ recipient mice. Expression of the plasminogen activators (PA), urokinase-type PA (u-PA) and tissue-type PA (t-PA), and expression of the matrix metalloproteinases (MMPs), MMP-3, MMP-9, MMP-12, and MMP-13, were significantly increased within 15 d of transplantation when cells actively migrate. These data indicate that plasmin proteolysis plays a major role in allograft arteriosclerosis by mediating elastin degradation, macrophage infiltration, media remodeling, medial smooth muscle cell migration, and formation of a neointima. (*J. Clin. Invest.* 1998. 102: 1788–1797.) Key words: plasmin proteolysis • atherosclerosis • transplant rejection • transgenic mice • transplantation

Introduction

Transplant arteriosclerosis constitutes a major limitation to long-term survival of cardiac and other solid organ transplants (1, 2). Therefore, a better understanding of the pathogenetic mechanisms of this life-threatening disease is mandated. Transplant arteriosclerosis differs from common atherosclerosis in its concentric lesions, reduced lipid accumulation, and faster progression. Mice with specific gene inactivations provide opportunities to dissect the pathogenic mechanisms of allograft disease, as transplantation models have been developed recently in the mouse (for review see reference 3).

The molecular mechanisms contributing to graft vascular disease remain poorly understood. It has been proposed that transplant arteriosclerosis is caused by an immune reaction of the recipient to donor graft antigens (4, 5). Indeed, inflammatory cells infiltrate the blood vessels of the transplanted organ and produce cytokines, growth factors, and chemotactic agents, such as IL-1, PDGF-B, basic FGF (FGF-2), IFN-γ, TGF-β, and TNF-α (4–6), that cause vascular smooth muscle cells to proliferate and migrate. However, for cells to migrate across anatomical borders (such as the elastic laminae), they require proteinases. For this purpose, vascular smooth muscle, endothelial, and inflammatory cells primarily depend on two proteinase systems, namely the plasminogen (Plg)[1] (7) and the matrix metalloproteinase (MMP) (5, 8) systems. However, their role in cellular migration and proliferation and in tissue remodeling during transplant arteriosclerosis has not been delineated conclusively.

The Plg system is composed of the inactive proenzyme Plg which is converted to plasmin by tissue-type (t-PA) or urokinase-type (u-PA) Plg activator (PA), the action of which is inhibited by PA inhibitors (PAIs) (9). Whereas t-PA is primarily involved in clot dissolution, u-PA has been implicated in pericellular proteolysis during cell migration and tissue remodeling. Recent gene targeting studies indicate that the Plg system plays a role in macrophage recruitment (10), arterial stenosis, atherosclerosis, aneurysm formation, skin and corneal wound healing, glomerulonephritis, and neovascularization (for review see reference 7).

MMPs can, in concert, degrade most components of the extracellular matrix of the vessel wall (8). Of the 20 MMPs identified to date, the interstitial collagenases (MMP-1 in humans and MMP-13 in mice), the stromelysins (MMP-3, MMP-7, and MMP-10), the gelatinases (MMP-2 and MMP-9), and an elastase (MMP-12) have been implicated in matrix degradation and cell migration in cardiovascular disorders (5, 8). Recently, we demonstrated that plasmin may be an important pathophysiological activator of the inactive zymogen pro-MMPs during

†This study is respectfully dedicated to the memory of Dr. E. Haber, who passed away during the course of this study.

Address correspondence to D. Collen, M.D., Ph.D., Center for Transgene Technology and Gene Therapy, Flanders Interuniversity Institute for Biotechnology, Campus Gasthuisberg, KU Leuven, Herestraat 49, B-3000 Leuven, Belgium. Phone: 32-16-345772; FAX: 32-16-345990; E-mail: desire.collen@med.kuleuven.ac.be

Received for publication 5 March 1998 and accepted in revised form 24 September 1998.

1. *Abbreviations used in this paper:* BrdU, 5′-bromo-2′-deoxyuridine; MMP, matrix metalloproteinase; PA, Plg activator; PAI, PA inhibitor; Plg, plasminogen; t-PA, tissue-type PA; u-PA, urokinase-type PA.

J. Clin. Invest.

0021-9738/98/11/1788/10 $2.00
Volume 102, Number 10, November 1998, 1788–1797
http://www.jci.org

atherosclerotic aneurysm formation (11) and arterial postinjury stenosis (12).

A mouse model of transplant arteriosclerosis has been developed (13), which is based on the grafting of a carotid artery from a donor mouse to the carotid artery of a recipient mouse, with histoincompatibility between donor and recipient in the H-2 region. Since many of the morphological features of the graft arterial disease in this model mimic those occurring after organ transplantation in humans, this model was used to evaluate whether the Plg system participates in the process of cellular migration and/or matrix remodeling during accelerated transplant arteriosclerosis. Therefore, carotid arteries from wild-type donors were transplanted into Plg-deficient ($Plg^{-/-}$) or wild-type recipients. The data indicate that graft arteriosclerosis is largely prevented in $Plg^{-/-}$ recipients because of the inability of macrophages to infiltrate the media and of smooth muscle cells to migrate into the intima. These findings may be useful for the design of antiproteolytic therapies against transplant arteriopathy.

Methods

Animals and transplantation. The model of carotid artery transplantation has been described (13). Male mice [B10.A(2R) strain ($H\text{-}2^{h2}$); The Jackson Laboratory, Bar Harbor, ME] were used as donors, and $Plg^{+/+}$ or $Plg^{-/-}$ littermates (14) with a mixed background of 75% C57BL/6 and 25% 129 ($H\text{-}2^{b}$) were used as recipients. In addition, C57BL/6 × 129 $Plg^{-/-}$ or $Plg^{+/+}$ carotid arteries were grafted to wild-type CBA ($H\text{-}2^{k}$) mice (Harlan Nederland BV, Zeist, The Netherlands).

Preparation of histological sections, morphometry, and immunohistochemistry. Grafts were harvested at 15 and 45 d after transplantation (groups of 8–10 $Plg^{+/+}$ and $Plg^{-/-}$ mice each). The proximal half of the transplanted loop was fixed in phosphate-buffered paraformaldehyde (1%) or in methyl-Carnoy fixative for 3 h, dehydrated, and embedded in paraffin. The distal half of the transplanted loop was immediately frozen in Tissue-Tek. 5-μm histological sections were made from the center of the graft towards the suture lines. Histochemical staining of elastin (Verhoeff's-Van Gieson) and collagen (Martius scarlet blue) and immunostaining of fibrin (antibody against murine fibrinogen; Nordic Immunology, Tilburg, The Netherlands), CD45 (pan-leukocyte marker; PharMingen, San Diego, CA), Mac-3 (macrophage marker; PharMingen), smooth muscle α-actin (smooth muscle cell marker; Sigma Chemical Co., St. Louis, MO), vWf (endothelial cell marker; Dakopatts, Copenhagen, Denmark), the proliferation marker 5'-bromo-2'-deoxyuridine (BrdU) (rat anti-BrdU mAb; Sera-Lab, Sussex, UK) were performed as described (14, 15). Morphometric measurements of cross-sectional areas, cell counts, and proliferation rates were performed on transverse sections of the artery using a computer-assisted image analysis system (Quantimet Q600 system; Leica, Nussloch, Germany) on four sections equally spaced, across the first 1,500 μm from the center of the graft, excluding the fragments at the suture lines. The average values per artery were used to determine the mean±SEM for the various measurements.

Expression of PAs and MMPs. In situ zymographic analysis of PA activities was performed on 7-μm cryosections using fibrin overlay gels (16). Lysis attributable to t-PA or u-PA activity was determined by inclusion of t-PA or u-PA specific IgGs (200 μg/ml), respectively. The amount of lysis (quantified using the Quantimet Q600 image analysis software) was expressed in square millimeters of lytic area.

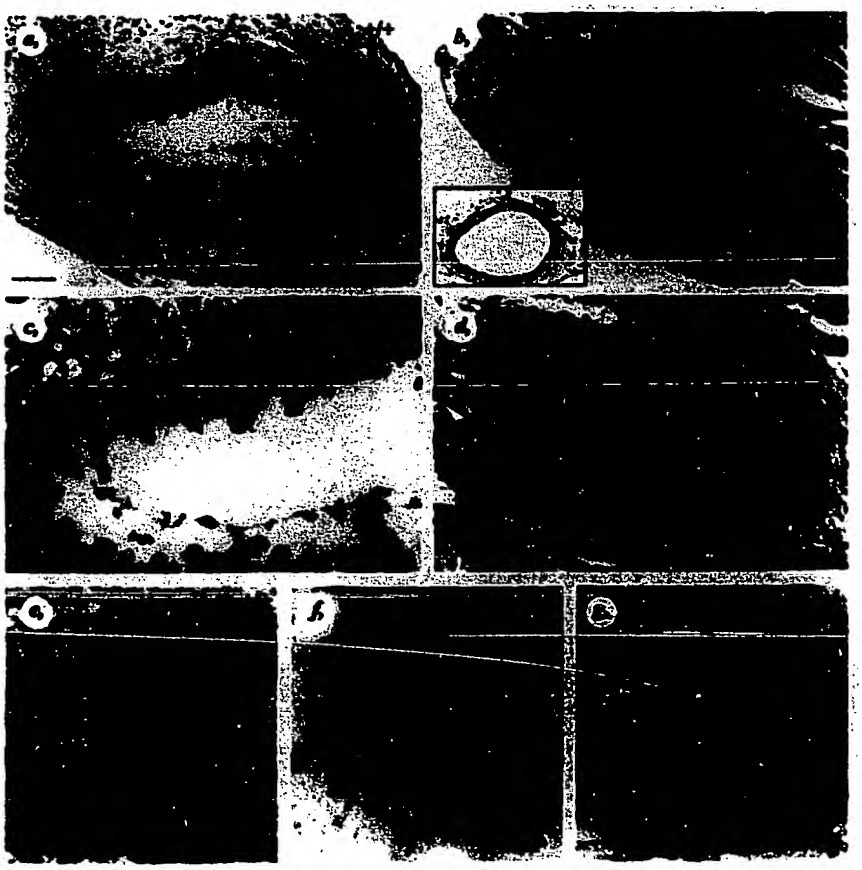


Figure 1. Light microscopic analysis of transverse sections through 15-d carotid grafts in $Plg^{+/+}$ (*a*, *c*, *e*, and *f*) and $Plg^{-/-}$ (*b*, *d*, and *g*) recipient mice, revealing a few intimal cell layers. In a control carotid artery (*b*, *inset*), the intima only consists of endothelial cells. Intimal cells accumulated underneath vWf-stained cells (*tailed arrows*) of the transplants (*e–g*). Note the more extensive graft adventitial cellular infiltration in the $Plg^{+/+}$ (*a*) than in the $Plg^{-/-}$ (*b*) recipient mice. The arrowheads indicate the internal elastic laminae and the arrows the external elastic laminae. Magnification bar is 300 μm in the inset of *b*; 50 μm in *a* and *b*; 25 μm in *c*, *d*, *e*, and *g*; and 10 μm in *f*.

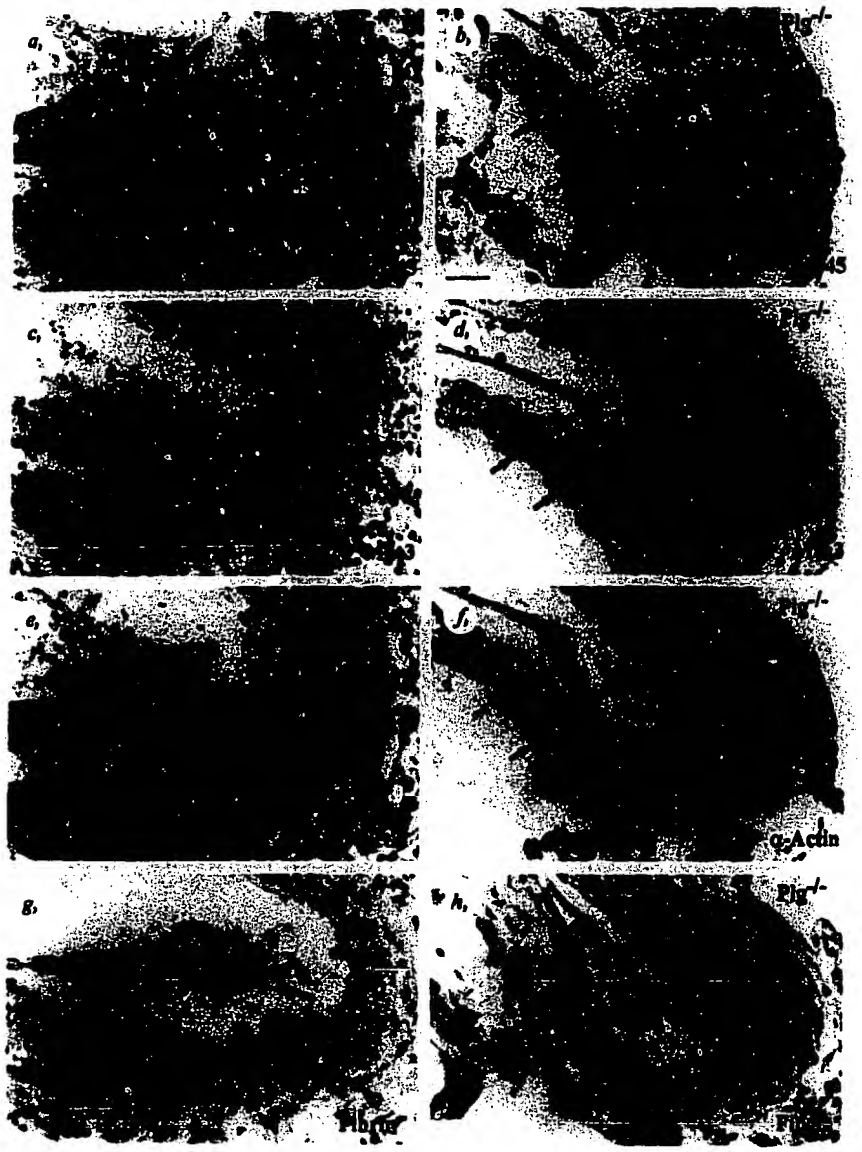


Figure 2. Immunostainings for the leukocyte marker CD45 (*a* and *b*) and the macrophage marker Mac3 (*c* and *d*) on transverse sections through the 15-d carotid grafts of $Plg^{+/+}$ (*a*, *c*, *e*, and *g*) and $Plg^{-/-}$ (*b*, *d*, *f*, and *h*) recipient mice revealed that the majority of the intimal cells represent leukocytes and macrophages in both genotypes. Immunostaining with the smooth muscle cell marker α-actin (*e* and *f*) revealed that most cells in the media of $Plg^{-/-}$ mice stained for α-actin, whereas the media of $Plg^{+/+}$ recipients was hypercellular and contained cells immunoreactive for α-actin, CD45, and Mac3. A fraction of medial cells remained unstained for all three cell markers, possibly representing proliferating and/or migrating smooth muscle cells (*tailed arrows*). Strongly stained fibrin-rich thrombi, infiltrated by leukocytes (*a*), were detected in the center of the lumen in grafts transplanted in $Plg^{+/+}$ (*g*) or $Plg^{-/-}$ (*h*) mice, whereas low levels of fibrin(ogen)-immunoreactivity (*tailed arrows*) were detectable in the subendothelial intima of transplants in $Plg^{+/+}$ (*g*) and $Plg^{-/-}$ (*h*) mice and scattered throughout the media in $Plg^{+/+}$ (*g*) recipients. The arrowheads indicate the internal elastic laminae and the arrows the external elastic laminae. Magnification bar is 25 μm in all panels.

Immunohistochemistry for t-PA, u-PA, and PAI-1 or for the MMPs, using previously characterized antibodies against murine t-PA, u-PA, PAI-1, MMP-3, MMP-9, MMP-12 (gift from S. Shapiro, Washington University, St. Louis, MO), and MMP-13 (provided by Y. Eeckhout, UCL, Brussels, Belgium) was performed as described previously (11, 12, 16, 17).

Statistical analysis. Experimental values were expressed as mean± SEM. Statistically significant differences between groups were calculated by the unpaired Student's *t* test or by the Mann-Whitney U test.

Results

Histological analysis. In control carotid arteries, the intima consisted of endothelial cells and the media of smooth muscle cells, whereas leukocytes were absent (Fig. 1 *b*, *inset*). Within 15 d after transplantation, a similar number of host-derived leukocytes infiltrated underneath the graft endothelium in both $Plg^{+/+}$ and $Plg^{-/-}$ recipients, constituting thereby a minimal neointima (Fig. 1, *a–d*), composed of vWf-immunoreactive endothelial cells (Fig. 1, *e–g*), $CD45^+$ leukocytes (Fig. 2, *a* and *b*), and Mac3-immunoreactive macrophages (Fig. 2, *c* and *d*). Immunostaining for fibrin(ogen) revealed strong staining in thrombi in the center of the lumen in both $Plg^{+/+}$ and $Plg^{-/-}$ recipients (Fig. 2, *g* and *h*). Upon analysis of serial sections, these thrombi did not appear to contact the endothelium, thereby differing from the mural thrombi incorporated within the subendothelial intima in $Plg^{-/-}$ recipients at day 45 after transplantation (see below). These thrombi may represent nonoccluding residual thrombi formed during surgical transplantation. Compared with the strong staining intensity of luminal fibrin-thrombi, much lower levels of fibrin(ogen)-immunoreactivity were detected within the media in $Plg^{+/+}$ mice and within the intima boarding the internal elastic lamina in both $Plg^{+/+}$ and $Plg^{-/-}$ hosts (Fig. 2, *g* and *h*).

Significant genotype-related differences were observed in the media and the adventitia between grafts in $Plg^{-/-}$ and $Plg^{+/+}$ mice. In $Plg^{+/+}$ recipients, the internal elastic lamina (and occasionally also the external elastic lamina) was fragmented and

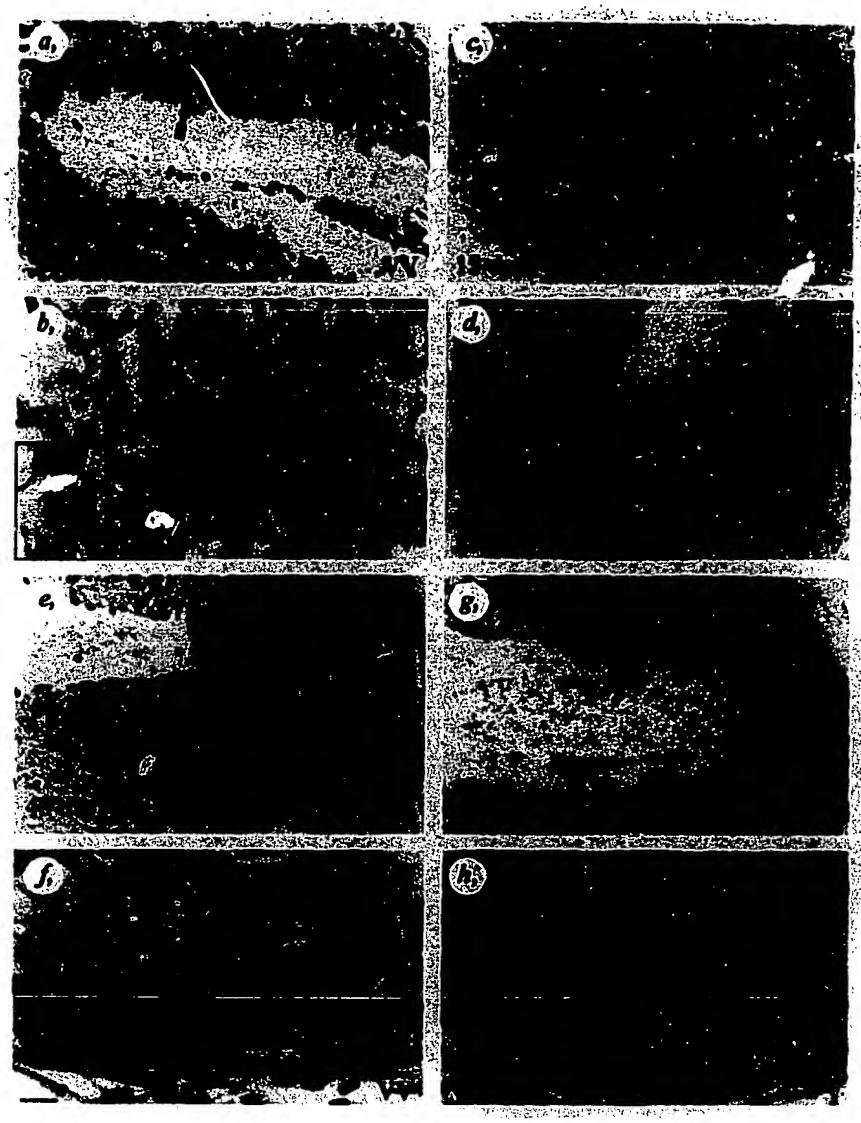


Figure 3. Verhoeff's Van Gieson staining of transverse sections through carotid transplants at 15 d (*a–d*) revealing small breaks (*tailed arrows*) in the internal and external elastic lamina in grafts in $Plg^{+/+}$ mice (*a* and *b*), through which macrophages migrated from the subendothelial space into the media (a migrating $Mac3^+$ macrophage is shown in the inset in *b*). In contrast, the elastic laminae and the media of the grafts in $Plg^{-/-}$ hosts (*c* and *d*) remained largely intact. At 45 d, the elastic laminae of the transplants in $Plg^{+/+}$ mice appeared very thin, elongated, and fragmented over large distances, with numerous major breaks (*e* and *f*), whereas elastic lamina degradation and disruption were less pronounced in the $Plg^{-/-}$ mice (*g* and *h*). Magnification bar is 25 μm in *a*, *c*, *e*, and *g*, and 10 μm in *b*, *d*, *f*, and *h*.

showed minor breaks (Fig. 3, *a* and *b*), through which leukocytes and macrophages infiltrated into the medial layers (Fig. 3 *b*, *inset*). In contrast, in $Plg^{-/-}$ mice, the elastic laminae remained structurally more intact, and macrophages largely failed to infiltrate the media (Fig. 3, *c* and *d*). Compared with the media of grafts in $Plg^{-/-}$ mice, which only contained α-actin positive smooth muscle cells (Fig. 2 *f*), the media in $Plg^{+/+}$ mice was hypercellular (Fig. 2 *e*), primarily due to the infiltration of $CD45^+$ and $Mac3^+$ leukocytes (Fig. 2, *a* and *c*). Some CD45- and Mac3-negative cells were oriented perpendicularly to the medial plane (Fig. 1 *c* and Fig. 2, *a* and *c*, and data not shown), and may represent smooth muscle cells migrating into the intima. Proliferating BrdU-positive cells in the $Plg^{+/+}$ mice were initially detected in the internal and external medial layers, which became first infiltrated by leukocytes from the intima or adventitia, respectively (data not shown). The middle layer of the media only contained proliferating cells by 45 d (not shown), suggesting that smooth muscle cell proliferation was attributable to mitogenic growth factors released by inflammatory cells infiltrated into the media (as previously suggested in other models) (4, 5). Infiltration of the adventitia by leukocytes (stained cells in Fig. 2, *a* and *b*) and fibroblasts (unstained cells in Fig. 2, *a* and *b*) was markedly greater in the allografts in $Plg^{+/+}$ (Fig. 1 *a* and Fig. 2 *a*) than in $Plg^{-/-}$ (Fig. 1 *b* and Fig. 2 *b*) recipient mice.

At 45 d after transplantation, a concentric neointima, consisting almost exclusively of strongly stained α-actin smooth muscle cells (Fig. 4, *a* and *e*) and containing abundant collagen (Martius scarlet blue staining) (Fig. 4 *h*), frequently occluded the entire lumen in $Plg^{+/+}$ mice (in five of nine mice). In contrast, in $Plg^{-/-}$ mice, the intima contained only a few α-actin smooth muscle cells (Fig. 4, *b* and *f*), scattered amid the more numerous CD45- (Fig. 4 *d*) and Mac3-immunoreactive leukocytes (not shown). Intimal collagen deposition was not or only minimally observed in arteries grafted into $Plg^{-/-}$ mice (Fig. 4 *j*). Beyond 15 d, fibrin deposits in the intima of grafts in $Plg^{-/-}$ mice (Fig. 4, *b* and *i*) were more frequent and abundant than in the intima in $Plg^{+/+}$ mice (Fig. 4 *g*). Interestingly, α-actin immunostaining was frequently reduced in the transplant media in $Plg^{-/-}$ mice at sites of elastic laminae fragmentation, where

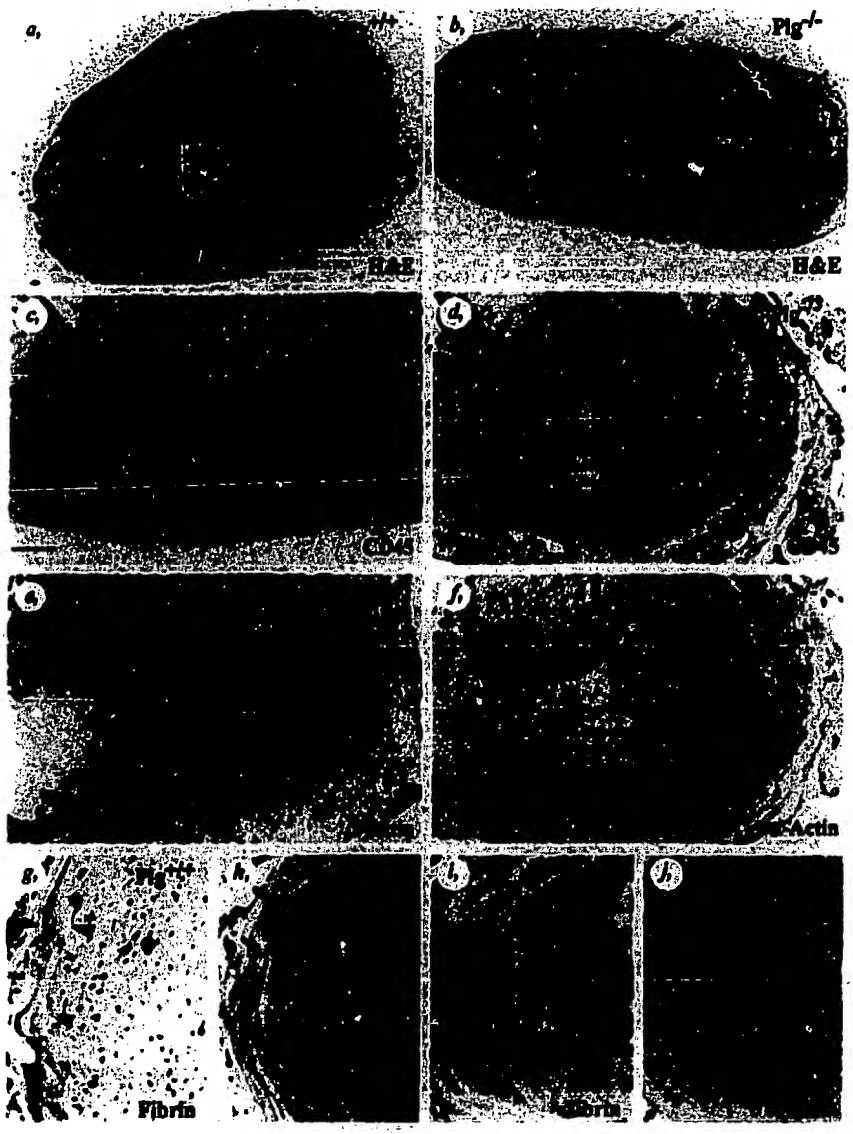


Figure 4. Light microscopic analysis of transverse sections through the carotid grafts of $Plg^{+/+}$ (*a*, *c*, *e*, *g*, and *h*) and $Plg^{-/-}$ (*b*, *d*, *f*, *i*, and *j*) recipient mice in 45-d transplants. A multilayered concentric neointima developed in allografts of $Plg^{+/+}$ recipients (*a*) whereas fewer cells were intermingled with mural thrombi in the intima in $Plg^{-/-}$ mice (*b*). $CD45^+$ leukocytes (*c* and *d*) accumulated in the intima of $Plg^{+/+}$ and $Plg^{-/-}$ mice and in the media of $Plg^{+/+}$ hosts, but only rarely in the media of $Plg^{-/-}$ recipients (only at sites where the internal elastic lamina was ruptured; *tailed arrows*). Smooth muscle cell α-actin positive cells were abundant in the intima of $Plg^{+/+}$ recipient mice (*e*) but rare in the intimal layers of $Plg^{-/-}$ mice (*f*), whereas they were largely absent in the media of $Plg^{+/+}$ mice (*e*) but still numerous in the media of $Plg^{-/-}$ hosts (*f*). Fibrin deposition occurred in acellular thrombi (*asterisk*, *b* and *i*) and in cell-rich lesions (*circle*, *b* and *i*) in the intimal layer of grafts in $Plg^{-/-}$ mice and was less abundantly detectable in the intima (*circle*, *g*) of $Plg^{+/+}$ mice (*j*). The media was replaced by cell debris and fibrous tissue in $Plg^{+/+}$ (*a*, *g*, and *h*) but not in $Plg^{-/-}$ (*b*, *i*, and *j*) recipients. The arrowheads indicate the internal elastic laminae and the arrows the external elastic laminae. Magnification bar is 50 μm in *a* and *b* and 25 μm in all other panels.

leukocytes had infiltrated the media (compare serial sections in Fig. 4, *d* and *f*). Since smooth muscle cells typically loose their α-actin immunoreactivity once they start to proliferate and migrate, these data may suggest that infiltrating leukocytes locally activate the smooth muscle cells in the media to proliferate (Fig. 4 *f*) and to migrate into the intima.

The genotypic differences in the media, present at 15 d, became more pronounced by 45 d. The elastic laminae in the grafts in $Plg^{+/+}$ hosts appeared thin, elongated, and fragmented over large distances, with numerous major breaks (Fig. 3, *e* and *f*). In contrast, elastic lamina degradation and disruption were much less pronounced in the $Plg^{-/-}$ mice (Fig. 3, *g* and *h*), suggesting that loss of Plg markedly reduced but did not completely prevent elastolysis (perhaps because of the involvement of other plasmin-independent matrix degrading proteinases). CD45- (Fig. 4 *c*) as well as Mac3-immunopositive cells (not shown) were numerous in the media in $Plg^{+/+}$ grafts, but only rarely detectable in the media in $Plg^{-/-}$ recipients (except at the rare sites of elastic lamina fragmentation) (Fig. 4 *d*). Conversely, α-actin positive smooth muscle cells were numerous in the media in $Plg^{-/-}$ arteries (Fig. 4 *f*), but largely absent in the media in $Plg^{+/+}$ mice (Fig. 4 *e*). The normal media was replaced by cell debris, tissue necrosis, and fibrin-rich matrix in $Plg^{+/+}$ (Fig. 4, *a* and *g*) but not in $Plg^{-/-}$ mice (Fig. 4, *b* and *i*). By 45 d, the marked adventitial infiltration by inflammatory cells (observed at 15 d) had largely disappeared in $Plg^{+/+}$ mice (Fig. 4 *a*), whereas only minimal inflammation and fibroblast accumulation were present in $Plg^{-/-}$ mice (Fig. 4 *b*).

Morphometric analysis. Intimal, medial, and adventitial areas were measured at 15 and 45 d after transplantation. In 15-d transplants, the intimal area of the grafts was somewhat larger in $Plg^{+/+}$ mice (0.010 ± 0.001 mm^2, $n = 8$) than in $Plg^{-/-}$ mice (0.006 ± 0.001 mm^2, $n = 8$, P = NS), but by 45 d, the intima was significantly larger in $Plg^{+/+}$ mice (0.069 ± 0.002 mm^2) than in $Plg^{-/-}$ mice (0.025 ± 0.004 mm^2, $n = 9$, $P < 0.001$ by unpaired t test). No significant genotypic differences were found in the medial areas at 15 d (0.041 ± 0.002 mm^2 in $Plg^{+/+}$ vs. 0.030 ± 0.004 mm^2 in $Plg^{-/-}$ mice, $n = 8$, P = NS), nor at 45 d after transplantation (0.047 ± 0.004 mm^2 in $Plg^{+/+}$ vs. 0.039 ± 0.003 mm^2 in $Plg^{-/-}$ mice, $n = 9$, P = NS). The adventitial area was

Table I. Cell Counts in the Medial and Intimal Layers of $Plg^{+/+}$ and $Plg^{-/-}$ Recipient Mice in Normal Carotid Artery and in Carotid Grafts at 15 and 45 d after Transplantation

		Intima		Media	
	Cell type	$Plg^{+/+}$	$Plg^{-/-}$	$Plg^{+/+}$	$Plg^{-/-}$
Control arteries	α-actin	0	0	120±14	110±11
	CD45	0	0	0	0
	Mac3	0	0	0	0
15-d transplant	α-actin	0	0	130±19	110±5
	CD45	50±9	51±9	17±6*	0.4±0.4
	Mac3	24±8	11±4	18±10*	0.3±0.2
45-d transplant	α-actin	170±27‡	12±7	5±2*	49±17
	CD45	43±12	48±14	23±7*	4±1
	Mac3	16±3*	33±6	17±5§	5±1

Cell counts were performed on four sections per transplant equally spaced throughout 1,500 μm starting from the center of the graft, which were then averaged. The data represent the mean±SEM of these averages in eight grafts per genotype and per time point. *$P < 0.05$, §$P < 0.005$; and ‡$P < 0.001$ by unpaired Student's t test versus $Plg^{-/-}$.

threefold larger in $Plg^{+/+}$ than in $Plg^{-/-}$ mice by 15 d (0.150±0.020 mm^2 in $Plg^{+/+}$ vs. 0.055±0.006 mm^2 in $Plg^{-/-}$, n = 8, $P < 0.001$ by unpaired Student's t test). However, by 45 d, the adventitial areas were comparable in both genotypes (0.062±0.007 mm^2 in $Plg^{+/+}$ vs. 0.058±0.011 mm^2 in $Plg^{-/-}$, n = 9, P = NS).

Cell counts. In control carotid arteries, the intima was devoid of leukocytes and smooth muscle cells, whereas the media consisted exclusively of α-smooth muscle actin positive cells (Table I). By day 15 after transplantation, a similar number of intimal leukocytes and macrophages and of medial α-actin positive smooth muscle cells was present in both genotypes, whereas there were significantly more $CD45^+$ and $Mac3^+$ cells in the media of grafts transplanted in $Plg^{+/+}$ than in $Plg^{-/-}$ recipients. By 45 d, the intima of the transplants in $Plg^{+/+}$ mice contained 13-fold more α-actin positive cells than in $Plg^{-/-}$ mice. Although a similar number of $CD45^+$ leukocytes was present in the intima in $Plg^{+/+}$ and $Plg^{-/-}$ mice, fewer Mac3-immunoreactive macrophages were present in the intima in $Plg^{+/+}$ than in $Plg^{-/-}$ recipients. The transplant media in $Plg^{+/+}$ recipients was almost devoid of α-actin positive smooth muscle cells, whereas α-actin smooth muscle immunoreactive cells were numerous in the transplant media in $Plg^{-/-}$ hosts. There were significantly more CD45- and Mac3-immunostained cells in the transplant media in $Plg^{+/+}$ than in $Plg^{-/-}$ hosts.

Cellular proliferation. Cellular proliferation was quantified by determining the percentage of BrdU-stained cells. By 15 d after transplantation, cellular proliferation rates were similar in the transplant intima in $Plg^{+/+}$ mice (21±5%) and in $Plg^{-/-}$ mice (20±9%; n = 8, P = NS), but significantly higher in the transplant media in $Plg^{+/+}$ mice (15±3%) than in $Plg^{-/-}$ mice (1.3±0.6%; n = 8, $P < 0.01$ vs. $Plg^{-/-}$ by Student's t test). By 45 d, corresponding rates in the transplant intima were 19±3% in $Plg^{+/+}$ mice and 8±3% (n = 9, P = NS) in $Plg^{-/-}$ mice, and, in the media, 12±3% in $Plg^{+/+}$ mice and 6±2% in $Plg^{-/-}$ mice (n = 9, P = 0.066).

Expression of PAs. The expression of t-PA, u-PA, and PAI-1 was immunocytochemically analyzed in control arteries (n = 3) and in wild-type arteries transplanted into $Plg^{+/+}$ recipients (n = 3). u-PA was undetectable in a control carotid artery; however by 15 d after transplantation, it was strongly induced in most medial and intimal cells, and in some cells scattered throughout the enlarged adventitia of the graft. By 45 d, u-PA expression still persisted in the media and the intima (Fig. 5, *a–c*). t-PA immunoreactivity was undetectable in the endothelium of a normal carotid artery, consistent with previous observations (16), but was detected in some medial, intimal, and adventitial cells by 15 d, and became restricted to a few cells in the intima and the media by 45 d (Fig. 5, *d–f*). Overall, fewer cells expressed t-PA than u-PA in the transplants. PAI-1 immunoreactivity that was minimal or undetectable in the media in control carotid arteries became induced in a small fraction of medial and intimal cells by 15 d, and was restricted to only a few intimal cells by 45 d (Fig. 5, *g–i*). Despite differences in cell composition in the grafts transplanted into $Plg^{+/+}$ and $Plg^{-/-}$ recipients, a comparable staining for PAs and PAI-1 was observed in 15 as well as in 45-d transplants (data not shown). This suggests that comparable levels for PAs and PAI-1 were present in different wound cells (smooth muscle cells, leukocytes, and fibroblasts) as also observed in injured arteries (16, 17). That expression of PAs and PAI-1 was comparable in both genotypes should not be surprising since a compensatory upregulation of PAs has never been detected in any of our previous mouse models. For example, u-PA was not increased in t-PA–deficient mice, and vice versa, t-PA activity was not changed in u-PA–deficient mice (18). In addition, PA expression and PAI-1 expression were similar in injured arteries in $Plg^{+/+}$ and in $Plg^{-/-}$ mice (19).

In situ zymography. u-PA and t-PA activities were measured by in situ zymography using fibrin overlays. Because of the high affinity for and activation by fibrin, t-PA has a greater specific activity than u-PA in this assay. t-PA–mediated lysis of the fibrin gel was evaluated by inclusion of u-PA immunoneutralizing antibodies (200 μg/ml) in the gel. Lysis over carotid sections from transplants after 15 d (0.88±0.18 mm^2, n = 4) or after 45 d (0.87±0.29 mm^2, n = 4) was slightly higher than lysis over sections of a control carotid artery (0.42±0.06 mm^2, n = 4, P = NS). u-PA–mediated lysis of the fibrin overlay was quantified after addition of neutralizing t-PA antibodies (200 μg/ml). Lysis over control carotid arteries was minimal (0.006±0.003 mm^2, n = 5), but was increased dramatically over the allograft sections by 15 d (0.186±0.035 mm^2, n = 5, $P <$ 0.001 vs. control) and by 45 d (0.219±0.066 mm^2, n = 5, $P <$ 0.001 vs. control) after transplantation. Lysis was completely inhibited by additional inclusion of neutralizing u-PA antibodies, indicating that the markedly increased lysis was essentially due to u-PA activity. Although the amount of u-PA–mediated lysis, attributable to two-chain u-PA (which is already active in the graft) or to single-chain u-PA (which can become activated during the assay procedure) cannot be distinguished, the data nevertheless suggest that expression and/or activity of u-PA are significantly increased in the allografts. These data are consistent with the enhanced u-PA immunostaining and with previous findings in the atherosclerotic aorta, in which net u-PA activity is significantly increased compared with control aorta (11). Similarly, u-PA expression is increased more than t-PA (as judged by the in situ activity measurements); however, somewhat different from the atherosclerotic model, t-PA expression (evidenced by immunostaining) is more widely spread in the transplant model. Whether this relates to a dif-

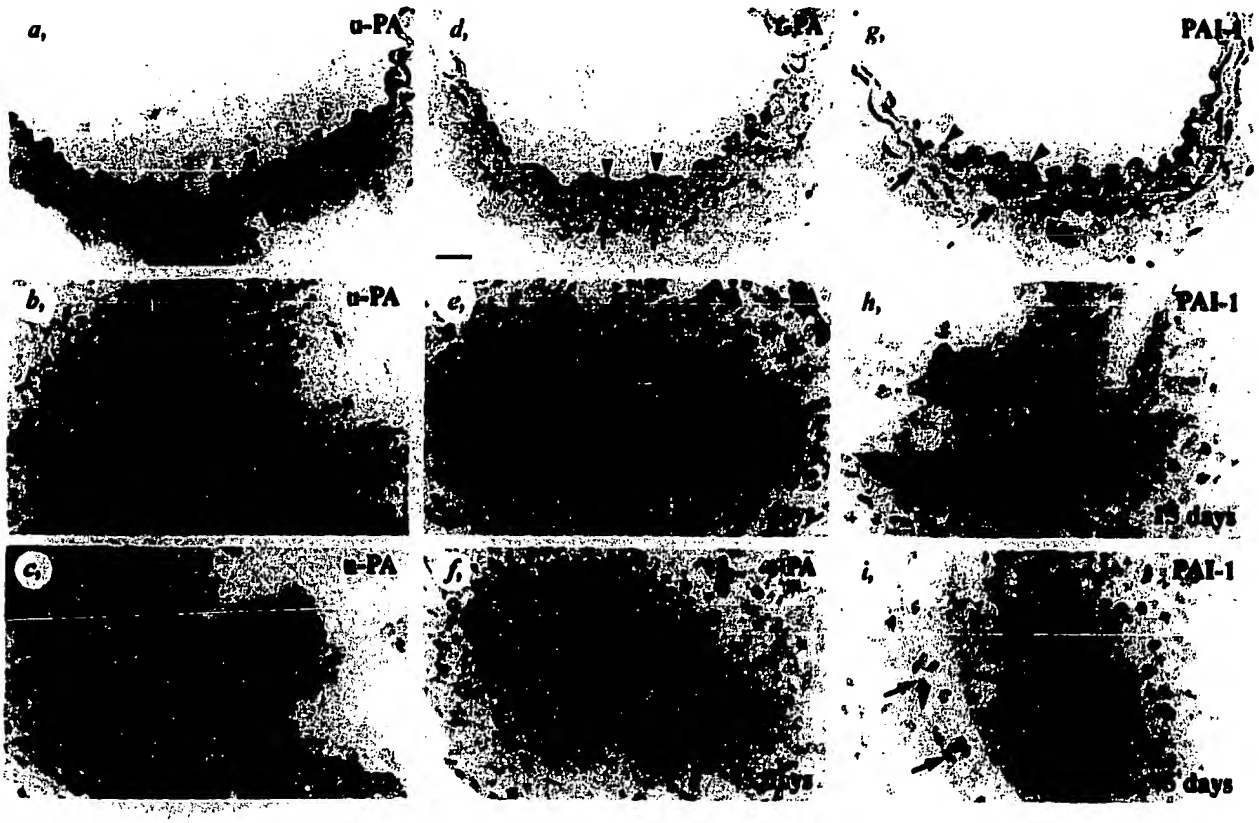


Figure 5. Immunohistochemical staining of u-PA (*a–c*), t-PA (*d–f*), and PAI-1 (*g–i*) in transverse sections of control carotid arteries and carotid arteries transplanted into $Plg^{+/+}$ mice. u-PA, t-PA, and PAI-1 expression was not detectable in normal carotid arteries (*a*, *d*, and *g*) but was significantly induced in the transplanted vessels at 15 (*b*, *e*, and *h*) and 45 d (*c*, *f*, and *i*) after transplantation. The arrowheads indicate the internal elastic laminae and the arrows the external elastic laminae. Magnification bar is 25 μm in all panels.

ferent microenvironment of cytokines/growth factors produced in the transplant versus in the atherosclerotic plaque remains to be determined. That t-PA fibrinolytic activity is only minimally increased (despite the more significant increase in t-PA immunostaining) probably suggests that activity of t-PA is inhibited more than that of u-PA. Similar observations were made during arterial intima formation (16). Nevertheless, we cannot exclude that t-PA plays a role in cellular migration and/or tissue remodeling during transplant arteriosclerosis.

Expression of metalloproteinases in carotid transplants. Immunostaining revealed that MMP-3, MMP-9, MMP-12, and MMP-13 were undetectable in control carotid arteries (Fig. 6, *a*, *c*, *e*, and *g*). Instead, in the 15-d transplants, MMP-3 and MMP-13 (which are generally more abundantly expressed by smooth muscle cells) were significantly induced throughout the media (MMP-3) (Fig. 6 *b*) or throughout the media and intima (MMP-13) (Fig. 6 *h*). MMP-9 and MMP-12 (typical macrophage-derived proteinases) were highly expressed in distinct cell clusters in all three layers of the grafted arteries (MMP-9) (Fig. 6 *d*), or in discrete groups of cells in the media or adventitia in the immediate vicinity of degraded elastin fibers (MMP-12) (Fig. 6 *f*). By 45 d, an essentially similar staining intensity was observed, although the number of immunoreactive cells was slightly reduced (in particular of MMP-13) (not shown). Comparable stainings were observed in the allografts of both $Plg^{+/+}$ and $Plg^{-/-}$ mice, although staining became only significantly induced after activation of the medial cells, as judged from lamina disruption and infiltration of leukocytes in the media (data not shown).

Role of host-versus-graft–derived Plg. These data indicate that Plg, circulating in the plasma of the recipient hosts, significantly influences the development of allograft arteriosclerosis. Although Plg is primarily produced by the liver, minimal amounts of Plg expression have been documented in extrahepatic tissues, including the brain (20). Therefore, the possible role of Plg, produced by the graft cells, was evaluated by transplanting carotid arteries from $Plg^{+/+}$ or $Plg^{-/-}$ donors into wild-type CBA mice. Histological analysis revealed that $Plg^{+/+}$ as well as $Plg^{-/-}$ donor allografts developed a large neointima with all of the typical histological hallmarks of adventitial remodeling, elastic lamina degradation, leukocyte infiltration into the media, medial cell proliferation, accumulation of smooth muscle-actin cells in the intima, and lack of fibrin-rich thrombi (Fig. 7, *a–d*) as observed in $Plg^{+/+}$ B10.A2R arteries, allografted into $Plg^{+/+}$ C57Bl6/129 mice. Furthermore, morphometric analysis indicated no genotype-related differences in the cross-sectional area of the intima (0.051 ± 0.010 mm^2 in $Plg^{+/+}$ vs. 0.056 ± 0.018 mm^2 in $Plg^{-/-}$ transplants, $n = 5$, P = NS), of the media (0.045 ± 0.003 mm^2 in $Plg^{+/+}$ vs. 0.066 ± 0.005 mm^2 in $Plg^{-/-}$ transplants, $n = 5$, P = NS), or of the adventitia (0.073 ± 0.006 mm^2 in $Plg^{+/+}$ vs. 0.091 ± 0.005 mm^2 in $Plg^{-/-}$ grafts; $n = 5$, P = NS) at 45 d after transplantation. Taken together, these results indicate that Plg, circulating in the plasma of the recipient, is permissive for the arteriopathy response in the transplants.

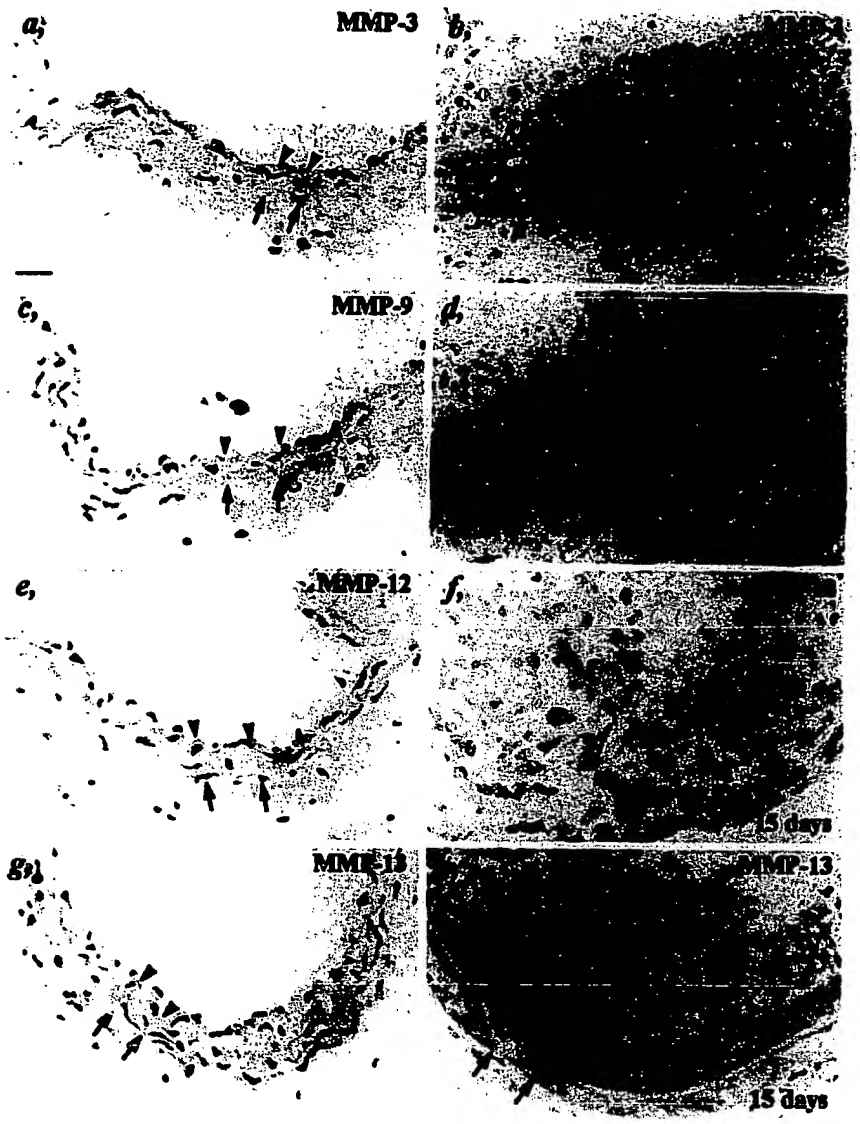


Figure 6. Immunohistochemical staining of MMP-3 (*a* and *b*), MMP-9 (*c* and *d*), MMP-12 (*e* and *f*), and MMP-13 (*g* and *h*) in transverse sections of control carotid arteries and arteries transplanted into $Plg^{+/+}$ mice. Expression of MMPs was undetectable in normal carotid arteries (*a*, *c*, *e*, and *g*), but significantly induced in the transplanted vessels at 15 d (*b*, *d*, *f*, and *h*). MMP expression was detected for MMP-3 in the proliferating media, for MMP-9 in all three cell layers of the transplants, for MMP-12 in the medial and adventitial layers, and for MMP-13 in the proliferating media and intima. The arrowheads indicate the internal elastic laminae and the arrows the external elastic laminae. Magnification bar is 25 μm in all panels.

Discussion

In previous reports, the role of the immune response and the consequences of hyperlipidemia on graft arterial disease have been studied (6, 21–24). This study demonstrates that plasmin proteolysis is also important for the development of graft arterial disease. Indeed, in the absence of circulating Plg, adventitial inflammation is diminished, degradation of the elastic laminae retarded, infiltration by macrophages into the media impaired, media necrosis reduced, proliferation of smooth muscle cells suppressed, and migration and accumulation of smooth muscle cells in the intima largely prevented. The involvement of the Plg system is further illustrated by the upregulated expression of PAs.

It has been proposed that leukocytes, once infiltrated into the media, produce several cytokines which trigger proliferation and migration of medial smooth muscle cells into the intima (4, 5, 23). Our observations that leukocyte infiltration into the media precedes smooth muscle cell proliferation and migration, and that smooth muscle cells in $Plg^{-/-}$ recipients accumulated at sites of internal elastic lamina rupture, where leukocytes had infiltrated the media, are consistent herewith. Whereas studies in other gene-inactivated mice demonstrated that the size of the allograft intima is largely determined by the accumulation of smooth muscle cells, and less so, of leukocytes, collagen, or lipid (6, 22–24), this study indicates that the neointima in $Plg^{-/-}$ recipients was only 3-fold smaller than in $Plg^{+/+}$ recipients, yet 13-fold fewer smooth muscle cells accumulated in their intima. However, the presence of fibrin deposits in the intima in $Plg^{-/-}$ recipients significantly contributed to the neointimal area in this genotype.

Plg deficiency impairs leukocyte recruitment, especially of monocytes, during inflammatory responses (10, 14). This is also true in the present model as evidenced by the reduced adventitial inflammation in grafts transplanted into $Plg^{-/-}$ recipients. Furthermore, plasmin is also important for leukocytes to infiltrate into the transplant media, and for smooth muscle cells to migrate into the intima of the allograft, similar to its role during atherosclerotic aneurysm formation and arterial stenosis (11, 16, 17, 19). Interestingly, however, comparable

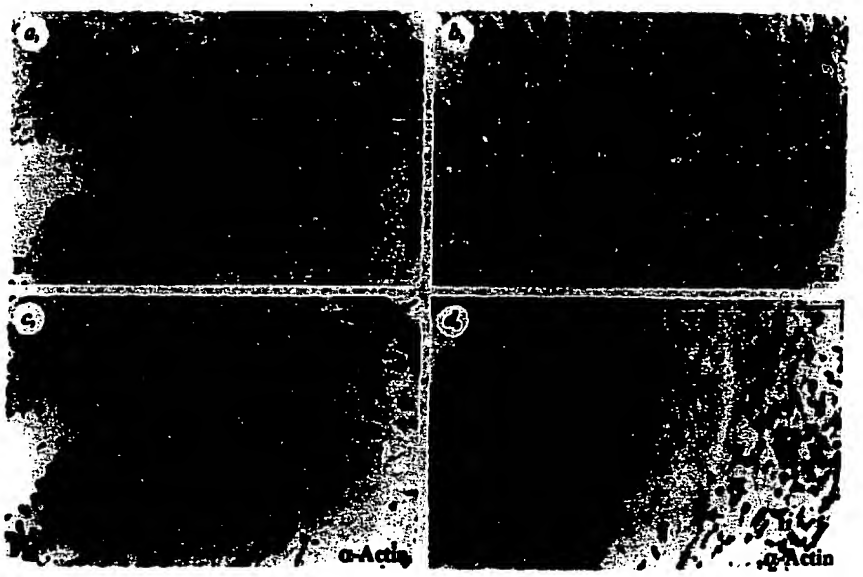


Figure 7. Light microscopic analysis of transverse sections through $Plg^{+/+}$ (*a* and *c*) and $Plg^{-/-}$ (*b* and *d*) carotid grafts transplanted into wild-type recipients, 45 d after transplantation. $Plg^{+/+}$ as well as $Plg^{-/-}$ allografts developed a large neointima within 45 d of transplantation (*a* and *b*), with all typical characteristics of adventitial remodeling, elastic lamina degradation, leukocyte infiltration into the media, medial cell proliferation, accumulation of α-smooth muscle cells in the intima (*c* and *d*), and lack of fibrin-rich thrombi as observed in wild-type carotid arteries grafted into $Plg^{+/+}$ mice. The arrowheads indicate the internal elastic laminae and the arrows the external elastic laminae. Magnification bar is 25 μm in all panels.

numbers of leukocytes infiltrated underneath the endothelium in both genotypes, indicating that subendothelial leukocyte infiltration does not critically depend on plasmin proteolysis, as also previously suggested by the similar growth of atherosclerotic fatty streaks in mice deficient in apo E, in apo E and u-PA, or in apo E and t-PA (11). Thus, plasmin plays a role in the degradation of some (elastin and collagen in the media) but not other (laminin and fibronectin in the subendothelial extracellular matrix) matrix components.

The Plg system may contribute to cell invasion in several manners: (*a*) via plasmin-mediated degradation of extracellular matrix components (9, 11); (*b*) via plasmin-mediated activation or liberation of chemotactic growth factors sequestered within the matrix, such as hepatocyte growth factor, basic fibroblast growth factor, vascular endothelial growth factor, or TGF-β1, which would attract leukocytes into the media or stimulate smooth muscle cells to emigrate into the intima (25–27); (*c*) via intracellular signaling mediated through the u-PA–u-PAR pathway (28); or (*d*) via an effect on cell–matrix interactions through a molecular interplay between u-PA, u-PAR, PAI-1, vitronectin, and integrins (29). Although direct evidence for the latter three hypotheses is not available, their possible involvement cannot be excluded either. It is likely that the role of plasmin in cell invasion is, at least in part, mediated by degradation of extracellular matrix components, such as fibrin. Fibrin deposits were detectable in the subendothelium in 15-d grafts transplanted in either $Plg^{+/+}$ or $Plg^{-/-}$ recipients, but much less frequently and abundantly as compared with those present in the intima of $Plg^{-/-}$ grafts or in the media of $Plg^{+/+}$ grafts at 45 d. Nevertheless, it is possible that the increased amounts of fibrin deposits in $Plg^{-/-}$ mice impede leukocyte infiltration into the media. That fibrin can be a barrier for infiltrating cells was demonstrated in a previous study whereby keratinocyte migration was impaired in $Plg^{-/-}$ mice and restored in $Plg^{-/-}$ mice that also lacked fibrinogen (30).

Whereas plasmin may degrade fibrin, laminin, and fibronectin directly, it is unable to directly degrade the insoluble elastin or collagen fibers and, therefore, most likely activates other proteinases. The present findings that MMP-3, MMP-9, MMP-12, and MMP-13 were induced after grafting and colocalized with PAs at sites of elastin degradation and smooth muscle cell emigration suggest that plasmin may activate downstream MMPs. In a recent study, plasmin production by macrophages in atherosclerotic plaques was also found to be responsible for proteolytic degradation of the elastic laminae, probably via activation of MMPs (11). However, direct proof for the causal involvement of MMPs in this process has to await similar studies in mice with inactivated MMP genes.

Media necrosis, associated with emigration of smooth muscle cells into the intima, was more severe in $Plg^{+/+}$ than in $Plg^{-/-}$ recipients. Gradual infiltration of the media by macrophages with loss of smooth muscle cells has been reported previously (31). Three alternative mechanisms have been proposed: (*a*) emigration of smooth muscle cells into the intima; (*b*) phenotypic switch of the smooth muscle cells to a macrophage-like phenotype; and (*c*) destruction of smooth muscle cells by the infiltrating macrophages. As discussed above, migration of smooth muscle cells into the intima was enhanced by Plg. Although the second hypothesis cannot be excluded, smooth muscle cell killing by infiltrating leukocytes is also a possible mechanism, as a similar loss of α-actin smooth muscle cells in the media appeared conditional on prior leukocyte infiltration into the atherosclerotic media (11). Macrophages could mediate the killing by releasing death signals (32), or alternatively, by disrupting the normal matrix architecture and cell–matrix interactions, triggering thereby anoikis of smooth muscle cells (33).

In conclusion, plasmin proteolysis plays an essential role in the development of graft arterial disease by controlling the infiltration of leukocytes in the media and, secondarily, the migration of smooth muscle cells into the intima. Previous studies in mutant or transgenic knockout mice with various defects in their immune response (6, 23) have indicated the importance of leukocytes in orchestrating the immune response in the allograft. Our study adds another dimension to the pathogenetic factors which contribute to this disease. That plasmin plays a pathophysiologically significant role in transplant arteriosclerosis, possibly by activation of pro-MMPs in vivo, might have

implications for the design of therapeutic strategies against graft vascular disease.

Acknowledgments

The authors thank Roger Lijnen (University of Leuven, Leuven, Belgium) for MMP-3 and MMP-9 antibodies; Steve Shapiro for MMP-12 antibodies; Yves Eeckhout for MMP-13 antibodies; A. Bouché, I. Cornelissen, M. De Mol, B. Hermans, A. Manderveld, A. Vandenbomen, and S. Wyns (CTG, Leuven, Belgium) for their expert help; and M. Deprez for artwork.

This work was supported by a grant from the Human Frontiers in Science Program (RG-363/95) to P. Carmeliet, V. Ploplis, L. Moons, E. Plow, and D. Collen, and a National Institutes of Health grant (HL-17964) to E. Plow.

References

1. Balk, A.H.M.M. 1992. Chronic heart graft rejection in the clinical setting. *In* Organ Transplantation: Long Term Results. L.C. Paul and K. Solez, editors. Marcel Dekker Inc., New York. 187–196.

2. Paul, L.C. 1994. Functional and histologic characteristics of chronic renal allograft rejection. *Clin. Transplant.* 8:319–323.

3. Carmeliet, P., L. Moons, and D. Collen. 1998. Mouse models of angiogenesis, arterial stenosis, atherosclerosis and hemostasis. *Cardiovasc. Res.* 39:8–33.

4. Russell, M.E., A.F. Wallace, W.W. Hancock, M.H. Sayegh, D.H. Adams, N.E. Sibinga, L.R. Wyner, and M.J. Karnovsky. 1995. Upregulation of cytokines associated with macrophage activation in the Lewis-to-F344 rat transplantation model of chronic cardiac rejection. *Transplantation.* 59:572–578.

5. Libby, P. 1995. Molecular bases of the acute coronary syndromes. *Circulation.* 91:2844–2850.

6. Nagano, H., R.N. Mitchell, M.K. Taylor, S. Hasegawa, N.L. Tilney, and P. Libby. 1997. Interferon-γ deficiency prevents coronary arteriosclerosis but not myocardial rejection in transplanted mouse hearts. *J. Clin. Invest.* 100:550–557.

7. Carmeliet, P., and D. Collen. 1996. Gene manipulation and transfer of the plasminogen and coagulation system in mice. *Semin. Thromb. Hemost.* 22: 525–542.

8. Dollery, C.M., J.R. McEwan, and A.M. Henney. 1995. Matrix metalloproteinases and cardiovascular disease. *Circ. Res.* 77:863–868.

9. Collen, D., and H.R. Lijnen. 1991. Basic and clinical aspects of fibrinolysis and thrombolysis. *Blood.* 78:3114–3124.

10. Ploplis, V.A., E.L. French, P. Carmeliet, D. Collen, and E.F. Plow. 1998. Plasminogen deficiency differentially affects recruitment of inflammatory cell population in mice. *Blood.* 91:1–6.

11. Carmeliet, P., L. Moons, R. Lijnen, M. Baes, V. Lemaître, P. Tipping, A. Drew, Y. Eeckhout, S. Shapiro, F. Lupu, and D. Collen. 1997. Urokinase-generated plasmin is a candidate activator of matrix metalloproteinases during atherosclerotic aneurysm formation. *Nat. Genet.* 17:439–444.

12. Lijnen, R., B. Van Hoef, F. Lupu, L. Moons, P. Carmeliet, and D. Collen. 1998. Function of the plasminogen/plasmin and matrix metalloproteinase systems after vascular injury in mice with targeted inactivation of fibrinolytic system genes. *Arterioscler. Thromb. Vasc. Biol.* 18:1035–1045.

13. Shi, C., M.E. Russell, C. Bianchi, J.B. Newell, and E. Haber. 1994. Murine model of accelerated transplant arteriosclerosis. *Circ. Res.* 75:199–207.

14. Ploplis, V.A., P. Carmeliet, S. Vazirzadeh, I. Van Vlaenderen, L. Moons, E.F. Plow, and D. Collen. 1995. Effects of disruption of the plasminogen gene on thrombosis, growth, and health in mice. *Circulation.* 92:2585–2593.

15. Carmeliet, P., L. Moons, J.M. Stassen, M. De Mol, A. Bouche, J.J. van den Oord, M. Kockx, and D. Collen. 1997. Vascular wound healing and neointima formation induced by perivascular electric injury in mice. *Am. J. Pathol.* 150:761–776.

16. Carmeliet, P., L. Moons, J.-M. Herbert, J. Crawley, F. Lupu, R. Lijnen, and D. Collen. 1997. Urokinase but not tissue plasminogen activator mediates arterial neointima formation in mice. *Circ. Res.* 81:829–839.

17. Carmeliet, P., L. Moons, R. Lijnen, S. Janssens, F. Lupu, D. Collen, and R.D.G. Gerard. 1997. Inhibitory role of plasminogen activator inhibitor-1 in arterial wound healing and neointima formation. A gene targeting and a gene transfer study. *Circulation.* 96:3180–3191.

18. Carmeliet, P., L. Schoonjans, L. Kieckens, B. Ream, J. Degen, R. Bronson, R. De Vos, J.J. van den Oord, D. Collen, and R.C. Mulligan. 1994. Physiological consequences of loss of plasminogen activator gene function in mice. *Nature.* 368:419–424.

19. Carmeliet, P., L. Moons, V. Ploplis, E. Plow, and D. Collen. 1997. Impaired arterial neointima formation in mice with disruption of the plasminogen gene. *J. Clin. Invest.* 99:200–208.

20. Tsirka, S.E., T.H. Bugge, J.L. Degen, and S. Strickland. 1997. Neuronal death in the central nervous system demonstrates a non-fibrin substrate for plasmin. *Proc. Natl. Acad. Sci. USA.* 94:9779–9781.

21. Russell, P.S., C.M. Chase, H.J. Winn, and R.B. Colvin. 1994. Coronary atherosclerosis in transplanted mouse hearts. II. Importance of humoral immunity. *J. Immunol.* 152:5135–5141.

22. Russell, P.S., C.M. Chase, and R.B. Colvin. 1996. Accelerated atheromatous lesions in mouse hearts transplanted to apolipoprotein-E-deficient recipients. *Am. J. Pathol.* 149:91–99.

23. Shi, C., W.S. Lee, Q. He, D. Zhang, D.L. Fletcher, Jr., J.B. Newell, and E. Haber. 1996. Immunologic basis of transplant-associated arteriosclerosis. *Proc. Natl. Acad. Sci. USA.* 93:4051–4056.

24. Shi, C., W.S. Lee, M.E. Russell, D. Zhang, D.L. Fletcher, J.B. Newell, and E. Haber. 1997. Hypercholesterolemia exacerbates transplant arteriosclerosis via increased neointimal smooth muscle cell accumulation: studies in apolipoprotein E knockout mice. *Circulation.* 96:2722–2728.

25. Saksela, O., and D.B. Rifkin. 1988. Cell-associated plasminogen activation: regulation and physiological functions. *Annu. Rev. Cell. Biol.* 4:93–126.

26. Ferrara, N., and T. Davis-Smyth. 1997. The biology of vascular endothelial growth factor. *Endocr. Rev.* 18:4–25.

27. Munger, J.S., J.G. Harpel, P.E. Gleizes, R. Mazzieri, I. Nunes, and D.B. Rifkin. 1997. Latent transforming growth factor-beta: structural features and mechanisms of activation. *Kidney Int.* 51:1376–1382.

28. Behrendt, N., E. Ronne, and K. Dano. 1995. The structure and function of the urokinase receptor, a membrane protein governing plasminogen activation on the cell surface. *Biol. Chem. Hoppe Seyler.* 376:269–279.

29. Chapman, H.A. 1997. Plasminogen activators, integrins, and the coordinated regulation of cell adhesion and migration. *Curr. Opin. Cell. Biol.* 9:714–724.

30. Bugge, T.H., K.W. Kombrinck, M.J. Flick, C.C. Daugherty, M.J. Danton, and J.L. Degen. 1996. Loss of fibrinogen rescues mice from the pleiotropic effects of plasminogen deficiency. *Cell.* 87:709–719.

31. Isik, F.F., T.O. McDonald, M. Ferguson, E. Yamanaka, and D. Gordon. 1992. Transplant arteriosclerosis in a rat aortic model. *Am. J. Pathol.* 141:1139–1149.

32. Lang, R., M. Lustig, F. Francois, M. Sellinger, and H. Plesken. 1994. Apoptosis during macrophage-dependent ocular tissue remodelling. *Development.* 120:3395–3403.

33. Frisch, S.M., and E. Ruoslahti. 1997. Integrins and anoikis. *Curr. Opin. Cell. Biol.* 9:701–706.

STIC-ILL

1633

From: Chen, Shin-Lin
Sent: Friday, January 14, 2000 10:34 AM
To: STIC-ILL
Subject: articles

Please provide the following articles by 1-19-2000. Thanks!
Serial No. 09/258,217.

L15 ANSWER 6 OF 8 MEDLINE DUPLICATE 1
AN 96159453 MEDLINE
DN 96159453
TI ***Elastin*** gene ***mutations*** in ***transgenic*** ***mice*** .
AU Sechler J L; Sandberg L B; Roos P J; Snyder I; Amenta P S; Riley D J; Boyd C D
CS Department of Surgery, UMDNJ-Robert Wood Johnson Medical School, New Brunswick 08903, USA.
NC HL 37438 (NHLBI)
HL 42798 (NHLBI)
HL 39869 (NHLBI)
SO CIBA FOUNDATION SYMPOSIUM, (1995) 192 148-65; discussion 165-71.
Journal code: D7X. ISSN: 0300-5208.
CY Netherlands
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199605

L15 ANSWER 7 OF 8 CAPLUS COPYRIGHT 2000 ACS
AN 1995:668015 CAPLUS
DN 123:48857
TI ***Elastin*** gene ***mutations*** in ***transgenic*** ***mice***
AU Sechler, Jan Louise
CS Rutgers the State U. of N.J. and U.M.D.N.J., New Brunswick, NJ, USA
SO (1994) 204 pp. Avail.: Univ. Microfilms Int., Order No. DA9511991
From: Diss. Abstr. Int. B 1995, 55(12) 5209
DT Dissertation
LA English

L15 ANSWER 8 OF 8 BIOSIS COPYRIGHT 2000 BIOSIS
AN 1994:401736 BIOSIS
DN PREV199497414736
TI Aortic disease in ***transgenic*** ***mice*** containing ***elastin*** gene ***mutations*** .
AU Sechler, Jan L.; Boyd, Charles D.
CS AMDNJ-Robert Wood Johnson Med. Sch., New Brunswick, NJ USA
SO Journal of Vascular Surgery, (1994) Vol. 20, No. 1, pp. 155-156.
ISSN: 0741-5214.
DT Article
LA English

MIC

L12 ANSWER 3 OF 4 SCISEARCH COPYRIGHT 2000 ISI (R)
AU Moons L; Shi C W; Ploplis V; Plow E; Haber E; Collen D (Reprint); Carmeliet P
TI Reduced transplant arteriosclerosis in plasminogen-deficient mice

2. White JV, Haas KS, Phillips SJ, Comerota AJ. Adventitial elastolysis is a primary event in aneurysm formation. J VASC SURG 1993;17:371-81.

AORTIC DISEASE IN TRANSGENIC MICE CONTAINING ELASTIN GENE MUTATIONS

Elastin is the major protein component of elastic fibers and is responsible for the resilient properties that elastic fibers confer on all elastic tissues.[1] Highly insoluble and extensively cross-linked, elastin is assembled from a family of isoforms of a soluble precursor protein collectively referred to as tropoelastin.[2] Tropoelastin isoforms are hydrophobic proteins with an average molecular weight of approximately 70 kd. This family of precursor proteins are synthesized from a single copy multiexon gene of approximately 40 kb in length.[2] The various isoforms of tropoelastin arise through a complex pattern of tissue-specific and developmentally regulated alternate usage of several exons within the tropoelastin gene.[3]

Although considerable information is now available concerning the biology and molecular biology of elastin in several vertebrate species, including human and rat, very little evidence is currently available concerning the existence and possible role of mutations in the tropoelastin gene in the pathogenesis of diseases of elastic tissue. It has been known for some time that altered elastin synthesis is associated with a variety of acquired and familial diseases ranging from skin disorders[4] to aortic aneurysms,[5] but only very recently has evidence for a direct causal role for elastin in the development of an elastic tissue disease been reported. Ewart and co-workers[6] and Olson et al.[7] have shown by linkage analysis that heritable forms of supravalvular aortic stenosis (SVAS) map to a locus on the long arm of human chromosome 7 (7q11.2) that includes the gene encoding tropoelastin. However, although some limited mutational analysis within the 7q11.2 locus has demonstrated the presence of large mutations involving all or part of the tropoelastin genes that are associated with the development of SVAS,[8] it is not yet established whether a mutation in the tropoelastin gene will result in either SVAS or any elastic tissue disorder.

Transgenic construction and expression

To directly address the hypothesis that mutations in the tropoelastin gene will cause an elastic tissue disorder, we have recently embarked on the construction of transgenic mice that contain minigene recombinants encoding rat tropoelastin, into which we had introduced several mutations. These minigene constructs contained a number of functional domains, including 3.2 kbp of the promoter region of the rat tropoelastin gene upstream of the most 5' exon, a single intron (intron 35), the complete 3'-untranslated region contained in exon 36 (the most 3' exon of the rat tropoelastin gene[9]), and varying exonic sequences covering coding domains represented in cDNA recombinants. With a total of four such minigene constructs containing either the complete coding sequence for rat tropoelastin or lacking exon sequences within the 5' or 3' end of the gene, a total of 600 fertilized mouse embryos were injected with aliquots of individual constructs. After implantation of injected embryos into pseudopregnant mice, a total of 113 live births were noted. By use of rat specific oligonucleotide primers, we demonstrated, using a polymerase chain reaction (PCR) assay on rat tail genomic DNA, that the minigene constructs were integrated in the genome of 28 of these founder mice. Moreover, with a reverse transcriptase-PCR assay, we were able to show that these rat tropoelastin minigene constructs were expressed as mRNA sequences present in total RNA isolated from a number of different tissues obtained from neonatal progeny in some of the founder mice. The expression of these minigene constructs appears to be tissue specific in the neonatal mice. With the same reverse transcriptase-PCR assay on total RNA from several tissues from adult transgenic mice, however, it was clear that the transgenes were not subject to the same developmental regulation imposed on the endogenous mouse tropoelastin gene. From these expression studies we were able to conclude that the DNA sequence elements necessary for tissue specific expression of the tropoelastin gene are located within the 3.2 kbp of promoter sequence present in all the minigene recombinants. The same domain, however, clearly lacks the cis-acting regulatory regions required for developmental regulation of the tropoelastin gene.

Phenotypic evaluation

Histopathologic analysis of several tissues from neonatal and adult transgenic animals that contained either the complete rat tropoelastin coding sequence or a minigene construct that lacked 13 exons (exons 19 to 31) at the 3' end of the gene has revealed altered elastic fiber deposition in aortic tissue in transgene animals expressing this truncated rat tropoelastin. An elastic fiber stain of the ascending aorta in one of these transgenic animals, for example, revealed a markedly hypoplastic elastic fiber and evidence for rupture of the thoracic aorta. This histopathologic evaluation, together with significantly reduced litter sizes and increased incidence of birth defects in progeny containing this truncated rat tropoelastin minigene, provides the very first evidence demonstrating that mutations in the tropoelastin gene will disrupt elastic fiber assembly and elastic tissue function.

Jan L. Sechler
Charles D. Boyd
AMDNJ–Robert Wood Johnson Medical School
New Brunswick, N.J.

We gratefully acknowledge the assistance of DNX (Princeton, N.J.) in preparing the transgenic mice. Supported by NIH grants HL 39869, HL 42798, HL 37438 and a Focused Giving Award from Johnson and Johnson.

REFERENCES

1. Rosenbloom J. Elastin: relation of protein and gene structure to disease. Lab Invest 1984;51:605-23.
2. Parks WC, Deak SB. Tropoelastin heterogeneity: implications for protein function and disease. Am J Respir Cell Mol Biol 1990;2:399-406.
3. Boyd CD, Pierce RA, Schwarzbauer JE, Doege K, Sandell LJ. Alternate exon usage is a commonly used mechanism for increasing coding diversity within genes coding for extracellular matrix proteins. Matrix 1993;13:457-69.
4. Uitto J, Shamban A. Heritable skin diseases with molecular defects in collagen or elastin. Derm Clin 1987;5:63-84.
5. Rizzo RJ, McCarthy WJ, Dixit SN, Lilly MP, Shively VP, Flinn WR., and Yao JST. Collagen types and matrix protein content in human abdominal aortic aneurysms. J VASC SURG 1989;10: 365-73.
6. Ewart AK, Morris CA, Ensing GJ, Loker J, Morre C, Leppert M, and Keating M. A human vascular disorder, supravalvular aortic stenosis, maps to chromosome 7. Proc Natl Acad Sci USA 1993;90:3226-30.

7. Olson TM, Michels VV, Lindor NM, Pastores GM, Weber JL, Schaid DJ, Driscoll DJ, Feldt RH, Thibodeau SN. Autosomal dominant supravalvular aortic stenosis: localization to chromosome 7. Hum Mol Genet 1993;2:869-73.
8. Curran ME, Atkinson DL, Ewart AK, Morris CA, Leppert M, Keating M. The elastin gene is disrupted by a translocation associated with supravalvular aortic stenosis. Cell 1993;73: 159-68.
9. Pierce RA, Alatawi A, Deak SB, Boyd CD. Elements of the rat tropoelastin gene associated with alternative splicing. Genomics 1992;12:651-8.

278757 File

STIC-ILL

1633

From: Chen, Shin-Lin
Sent: Friday, January 14, 2000 10:34 AM
To: STIC-ILL
Subject: articles

Please provide the following articles by 1-19-2000. Thanks!
Serial No. 09/258,217.

NO 1/14

L15 ANSWER 6 OF 8 MEDLINE DUPLICATE 1
AN 96159453 MEDLINE
DN 96159453
TI ***Elastin*** gene ***mutations*** in ***transgenic*** ***mice*** .
AU Sechler J L; Sandberg L B; Roos P J; Snyder I; Amenta P S; Riley D J; Boyd C D
CS Department of Surgery, UMDNJ-Robert Wood Johnson Medical School, New Brunswick 08903, USA.
NC HL 37438 (NHLBI)
HL 42798 (NHLBI)
HL 39869 (NHLBI)
SO CIBA FOUNDATION SYMPOSIUM, (1995) 192 148-65; discussion 165-71.
Journal code: D7X. ISSN: 0300-5208.
CY Netherlands
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199605

ZGR-00011888 79

L15 ANSWER 7 OF 8 CAPLUS COPYRIGHT 2000 ACS
AN 1995:668015 CAPLUS
DN 123:48857
TI ***Elastin*** gene ***mutations*** in ***transgenic*** ***mice***
AU Sechler, Jan Louise
CS Rutgers the State U. of N.J. and U.M.D.N.J., New Brunswick, NJ, USA
SO (1994) 204 pp. Avail.: Univ. Microfilms Int., Order No. DA9511991
From: Diss. Abstr. Int. B 1995, 55(12) 5209
DT Dissertation
LA English

L15 ANSWER 8 OF 8 BIOSIS COPYRIGHT 2000 BIOSIS
AN 1994:401736 BIOSIS
DN PREV199497414736
TI Aortic disease in ***transgenic*** ***mice*** containing ***elastin*** gene ***mutations*** .
AU Sechler, Jan L.; Boyd, Charles D.
CS AMDNJ-Robert Wood Johnson Med. Sch., New Brunswick, NJ USA
SO Journal of Vascular Surgery, (1994) Vol. 20, No. 1, pp. 155-156.
ISSN: 0741-5214.
DT Article
LA English

L12 ANSWER 3 OF 4 SCISEARCH COPYRIGHT 2000 ISI (R)
AU Moons L; Shi C W; Ploplis V; Plow E; Haber E; Collen D (Reprint); Carmeliet P
TI Reduced transplant arteriosclerosis in plasminogen-deficient mice

Elastin gene mutations in transgenic mice

Jan L. Sechler§, Lawrence B. Sandberg‡, Philip J. Roos‡, Ida Snyder, Peter S. Amenta*, David J. Riley† and Charles D. Boyd

Departments of Surgery, Pathology and †Medicine, UMDNJ-Robert Wood Johnson Medical School, New Brunswick, NJ 08903 and ‡Department of Pathology, Jerry L Pettis Memorial Veterans Hospital, Loma Linda University, Loma Linda, CA 92357, USA*

Abstract. We have constructed several rat tropoelastin minigene recombinants encoding the complete sequence of rat tropoelastin, two isoforms of rat tropoelastin and a truncated tropoelastin lacking the domains encoded by exons 19–31 of the rat gene. Coding and non-coding domains in all these recombinants were placed under the transcriptional control of 3 kb of the promoter domain of the rat tropoelastin gene. These minigenes were used to prepare a total of 28 separate founder lines of transgenic mice. A species-specific reverse-transcriptase polymerase chain reaction (RT-PCR) assay was established to demonstrate the synthesis of rat and mouse tropoelastin mRNA in several tissues obtained from both neonatal and adult transgenic mice. Thermolytic digestion of insoluble elastin isolated from several neonatal mouse tissues revealed the presence of rat tropoelastin peptides in progeny from all those founder mice in which detectable levels of rat tropoelastin mRNA were noted. Phenotypic and histopathological assessment of transgenic and non-transgenic animals revealed the development of two diverse elastic tissue disorders. The progeny of two separate founder lines overexpressing the rat tropoelastin isoform lacking exon 33, developed an emphysematous phenotype in early adulthood. In contrast, transgenic mice, in which expression of the truncated rat tropoelastin minigene lacking exons 19–31 had been observed, died of a ruptured ascending aortic aneurysm. Tropoelastin gene mutations, therefore, will result in heritable disorders of elastic tissue. Moreover, different mutations in the tropoelastin gene will be responsible for very different abnormalities in elastic tissue function.

1995 The molecular biology and pathology of elastic tissues. Wiley, Chichester (Ciba Foundation Symposium 192) p 148–171

Elastic fibres are extracellular matrix structures responsible for the properties of resilience and elastic recoil in all elastic tissues (Parks & Deak 1990, Rosenbloom 1984). There are two morphological components to elastic fibres,

§Present address: Department of Molecular Biology, Princeton University, Pinceton, NJ 08544, USA.

ı transgenic mice

J. Roos‡, Ida Snyder, Peter S.

:ine, UMDNJ-Robert Wood
903 and ‡Department of
pital, Loma Linda

tropoelastin minigene recombinants
tropoelastin, two isoforms of rat
lacking the domains encoded by
1 non-coding domains in all these
scriptional control of 3 kb of the
;ene. These minigenes were used to
of transgenic mice. A species-specific
ion (RT-PCR) assay was established
ouse tropoelastin mRNA in several
adult transgenic mice. Thermolytic
ım several neonatal mouse tissues
peptides in progeny from all those
rat tropoelastin mRNA were noted.
nt of transgenic and non-transgenic
diverse elastic tissue disorders. The
;pressing the rat tropoelastin isoform
ıus phenotype in early adulthood. In
on of the truncated rat tropoelastin
ıerved, died of a ruptured ascending
ıns, therefore, will result in heritable
ıt mutations in the tropoelastin gene
nalities in elastic tissue function.

of elastic tissues. Wiley, Chichester
'1

res responsible for the properties
c tissues (Parks & Deak 1990,
gical components to elastic fibres,

y, Princeton University, Pinceton, NJ

the microfibrillar component and the amorphous component (Mecham & Heuser 1991). The microfibrillar component is made up of 10–12 nm microfibrils that are composed of at least seven different glycoproteins, the best characterized of which are two genetically distinct 340 kDa glycoproteins called fibrillins (Gibson et al 1991, Lee et al 1991). Elastin constitutes the amorphous component of elastic fibres and is assembled on the microfibrillar scaffold from a soluble precursor, tropoelastin (Indik et al 1990). Over the last five years, extensive sequence analysis of cDNA and genomic DNA recombinants encoding tropoelastin from several phylogenetically diverse species has revealed that tropoelastin is indeed a family of hydrophobic proteins, all approximately 70 kDa and synthesized from a single-copy, multiexon gene by extensive alternate usage of several exons (Indik et al 1990, Boyd et al 1991, Pierce et al 1990). A typical isoform of tropoelastin is characterized by alternating hydrophobic domains (rich in glycine, valine and proline) and alanine-rich, lysine-containing sequences. These lysine-containing regions serve as a substrate for the formation of lysine-derived cross-links (desmosines and isodesmosines) that are formed during the assembly of elastin (Rosenbloom 1984). The formation of desmosine and isodesmosine cross-links is catalysed by an elastic fibre-associated enzyme, lysyl oxidase (Kagan 1986). Both the hydrophobic domains and the desmosine cross-links in elastin are necessary for the property of elastic recoil.

There are a variety of disorders characterized by abnormal elastin synthesis and a concomitant deposition of aberrant elastic fibres. Common, multifactorial diseases such as hypertension (Mecham et al 1987, Deyl et al 1985, Iredale et al 1989), atherosclerosis (Kramsch & Hollander 1973), actinic elastosis (Uitto et al 1989) and even some forms of breast cancer (Glaubitz et al 1984) involve increased accumulation of elastin and an associated deposition of morphologically atypical elastic fibres. Several, less common, heritable diseases are also characterized by an aberrant deposition of elastic fibres associated with either increased or decreased accumulation of elastin. For example, abnormal deposition of elastic fibres is characteristic of Marfan's syndrome (Hollister et al 1990) and supravalvular aortic stenosis (SVAS) (O'Connor et al 1985). Cutis laxa is a heritable cutaneous disorder characterized by reduced elastin synthesis (Olsen et al 1988). In contrast, pseudoxanthoma elasticum and the Buschke–Ollendorff syndromes are examples of heritable skin diseases in which an increased deposition of elastin has been demonstrated (Neldner 1988, Uitto & Shamban 1987).

In all of the rare and more common diseases of elastic tissue, the presence of mutations in genes responsible for the synthesis of elastin fibre proteins has been causally implicated. However, only in Marfan's syndrome have mutations in a fibrillin gene been identified that are clearly responsible for the pathogenesis of this disease (Dietz et al 1991). Although several recent reports of large mutations involving the tropoelastin gene have been described in

patients with SVAS (Curran et al 1993, Ewart et al 1994), there is no evidence to date that unambiguously shows that tropoelastin gene mutations will result in any elastic tissue phenotype.

Transgenic mice have been used extensively in the past to show that gene mutations will result in the pathogenesis of a wide range of heritable diseases (Jaenisch 1988, Palmiter & Brinster 1986). Recently, for example, mutations in the gene encoding type X collagen have been introduced into transgenic mice and have been shown to be responsible for the development of skeletal deformities in transgenic progeny (Jacenko et al 1993). Using a similar approach, in this paper we describe the construction of four rat tropoelastin minigenes, the introduction of these recombinants into transgenic mice and a biosynthetic, phenotypic and histopathological examination of mouse elastic tissue synthesizing normal and aberrant rat tropoelastin. The results demonstrate, for the first time, that mutations in the tropoelastin gene will cause an elastic tissue disorder. The diverse phenotypes that develop as a consequence of different tropoelastin gene mutations, moreover, establish an important precedent for a role for mutations in the tropoelastin gene in analogous human disorders of elastic tissue, including SVAS.

Results and discussion

Elastin is a polymer assembled from one or more isoforms of a monomeric subunit (Mecham & Heuser 1991). Our reasoning behind the construction of several founder strains of transgenic mice was that the introduction of an exogenous tropoelastin gene containing a mutation would result in the synthesis of abnormal tropoelastin monomers that would be incorporated into elastin together with the normal, endogenous mouse tropoelastin. If the exogenous tropoelastin is expressed at high enough levels and if the domain of tropoelastin affected by the mutation is important to elastin synthesis, then disruption of elastic fibre assembly and subsequent elastic tissue function should be apparent. In other words, the synthesis of the endogenous mouse tropoelastin should not mask a phenotype resulting from a mutation introduced into a tropoelastin gene. Indeed, the synthesis of endogenous, normal tropoelastin may be an important prerequisite to maintaining a non-lethal phenotype.

In preparation for the development of several founder transgenic animals, we prepared four constructs using our previously characterized cDNA and genomic DNA recombinants (Pierce et al 1990, 1992, Alatawi 1994) encoding rat tropoelastin (Fig. 1). All four recombinants contained approximately 3 kb of genomic DNA upstream of exon 1 of the rat tropoelastin gene. By DNA sequencing and expression studies (using the chloramphenicol acetyltransferase [CAT] reporter gene), we have shown that this 3 kb fragment contained many of the promoter elements necessary for the initiation and possible regulation of transcription of the rat tropoelastin gene (Sechler et al 1995a). This promoter

art et al 1994), there is no evidence
oelastin gene mutations will result

ely in the past to show that gene
a wide range of heritable diseases
ecently, for example, mutations in
n introduced into transgenic mice
for the development of skeletal
co et al 1993). Using a similar
nstruction of four rat tropoelastin
binants into transgenic mice and a
ical examination of mouse elastic
t rat tropoelastin. The results
ions in the tropoelastin gene will
se phenotypes that develop as a
mutations, moreover, establish an
ions in the tropoelastin gene in
, including SVAS.

r more isoforms of a monomeric
soning behind the construction of
was that the introduction of an
tation would result in the synthesis
ould be incorporated into elastin
se tropoelastin. If the exogenous
s and if the domain of tropoelastin
astin synthesis, then disruption of
issue function should be apparent.
ous mouse tropoelastin should not
on introduced into a tropoelastin
, normal tropoelastin may be an
-lethal phenotype.
veral founder transgenic animals,
viously characterized cDNA and
90, 1992, Alatawi 1994) encoding
nts contained approximately 3 kb
e rat tropoelastin gene. By DNA
chloramphenicol acetyltransferase
is 3 kb fragment contained many
itiation and possible regulation of
chler et al 1995a). This promoter

domain was followed by rat tropoelastin cDNA sequence corresponding to coding domains within exons 1–35. A single intron (intron 35) from the rat tropoelastin gene was introduced downstream of the cDNA sequence, followed by exon 36 which contained the termination codon and the complete 3′ untranslated domain.

The first of these minigene constructs (Tropoprom-1) contained the complete coding sequence for rat tropoelastin. Tropoprom-2 was identical to Tropoprom-1 except it lacked the cDNA sequence encoded by exon 33. Tropoprom-3 lacked the coding domain complementary to exons 13–15. The final construct, Tropoprom-4, lacked the coding sequence contained within exons 19–31 (Fig. 1).

Tropoprom-1 is essentially a control construct that was developed to establish that a normal elastic fibre will develop as a chimaera of mouse tropoelastin and the predominant, constitutively spliced isoform of rat tropoelastin. Tropoprom-2 and Tropoprom-3 should result in the synthesis of alternatively spliced isoforms of rat tropoelastin that lack exon 33 and exons 13–15, respectively. If the developmentally regulated and tissue-specific pattern of alternate usage of exon 33 (Heim et al 1991) is important to elastic fibre assembly, then the appearance of abnormal amounts of the tropoelastin isoform lacking the domain encoded by exon 33 should influence elastin assembly at least in some tissues. Similarly, Tropoprom-3 (lacking exons 13–15) should, if expressed in transgenic mice, also test the functional significance of splice variants of tropoelastin that lack the amino acid sequence encoded by these three exons (Pierce et al 1990). Tropoprom-4 contains a mutation lacking 13 exons at the 3′ end of the rat tropoelastin gene. If a disruption of coding sequence within the tropoelastin gene (not an aberration of synthesis of the pattern of tropoelastin isoforms) will result in an elastic tissue disorder, expression of this large mutation in the Tropoprom-4 construct should result in an elastic tissue pathology.

These four rat tropoelastin minigene constructs were separately injected into the male pronuclei of approximately 600 fertilized mouse embryos. The injected embryos were then implanted into pseudopregnant mice. A total of 113 live births were recorded. Using mouse tail genomic DNA and a PCR assay specific for the rat minigene recombinants, we demonstrated that 28 of these 113 founder mice contained the rat constructs integrated into genomic DNA. Four founder mice contained Tropoprom-1, 11 animals contained Tropoprom-2, 10 transgenic mice had integrated Tropoprom-3 into genomic DNA and three founder mice contained Tropoprom-4.

Expression of rat tropoelastin minigenes in transgenic mice

Founder transgenic mice (F_0) were bred with normal mice and tail clip DNA from the resulting progeny (F_1) was analysed for the incorporation of the rat

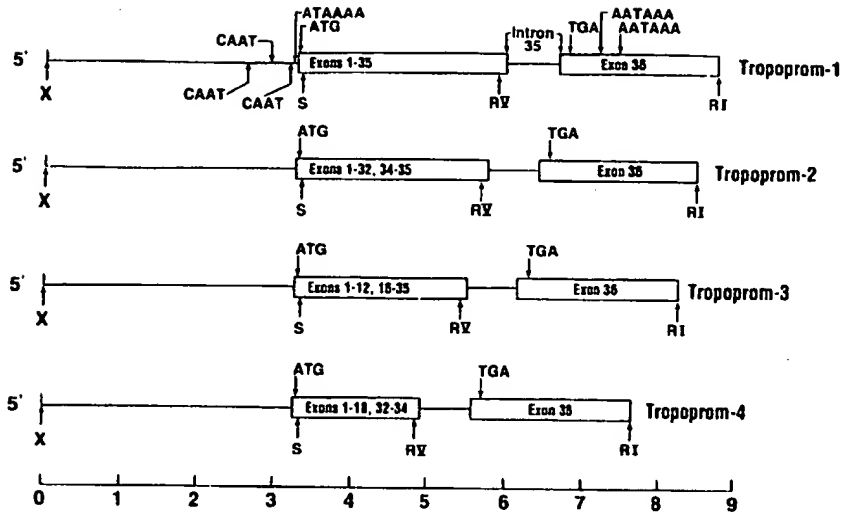
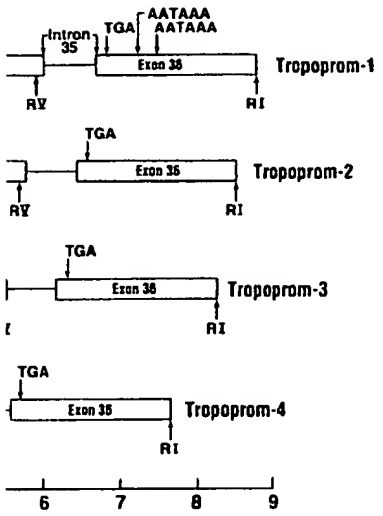


FIG. 1. The rat tropoelastin minigene contructs. Four minigene constructs (referred to as Tropoprom 1–4) were assembled from a combination of previously characterized rat tropoelastin cDNA and genomic DNA recombinants (Alatawi 1994, Pierce et al 1990, 1992). Each minigene contained 3.2 kb of the promoter domain 5′ of exon 1. This region is indicated as an *Xho*l (X) and *Sau*3A1 (S) restriction fragment which also included the ATG initiation codon. The positions of a TATA box motif (ATAAA) and three CAAT boxes located within this restriction fragment are also indicated. These *cis*-acting consensus sequences are present in all the Tropoprom constructs but are indicated only in the diagram of the Tropoprom-1 recombinant. Each Tropoprom construct also contains a single intron (intron 35) and the entire exon 36. Exon 36 encodes the cysteine-containing C-terminus of tropoelastin, a termination codon and a 3′ untranslated domain that includes two polyadenylation signals (AATAAA). This region of each Tropoprom construct was isolated as an *Eco*RV (RV)–*Eco*RI (RI) restriction fragment from a previously characterized genomic DNA recombinant. Each Tropoprom construct contained different exon domains represented in several rat tropoelastin cDNA recombinants. Tropoprom-1 contains a continuous cDNA sequence derived from exons 1–35. Tropoprom-2 contains the identical cDNA sequence but lacks the DNA sequence encoded by exon 33. Coding sequence derived from exons 13–15 is missing in Tropoprom-3. Exons 19–31 are absent in the Tropoprom-4 recombinant.

transgene into genomic DNA. Transgenic F_1 animals, hemizygous for the integrated copy or copies of the transgene were then used in a series of brother–sister matings to generate an F_2 generation, 25% of which will be homozygous for the integrated transgenes. Confirmed homozygous F_2 transgenic animals and, where necessary, hemizygous F_1 and F_2 transgenic progeny were then used to determine both trangsene copy number and levels of expression of the integrated rat tropoelastin minigene constructs.

Southern blot analysis of transgenic mouse tail genomic DNA, together with the appropriate copy number calibration standards derived from the rat



ts. Four minigene constructs (referred
ɔmbination of previously characterized
ɔmbinants (Alatawi 1994, Pierce et al
he promoter domain 5′ of exon 1. This
1 (S) restriction fragment which also
s of a TATA box motif (ATAAA) and
ɔn fragment are also indicated. These
ıll the Tropoprom constructs but are
om-1 recombinant. Each Tropoprom
35) and the entire exon 36. Exon 36
opoelastin, a termination codon and a
adenylation signals (AATAAA). This
ated as an *EcoRV* (RV)–*EcoRI* (RI)
zed genomic DNA recombinant. Each
ı domains represented in several rat
contains a continuous cDNA sequence
the identical cDNA sequence but lacks
sequence derived from exons 13–15 is
ıt in the Tropoprom-4 recombinant.

: F_1 animals, hemizygous for the
ıe were then used in a series of
generation, 25% of which will be
ıes. Confirmed homozygous F_2
hemizygous F_1 and F_2 transgenic
angsene copy number and levels of
ı minigene constructs.
e tail genomic DNA, together with
standards derived from the rat

minigene constructs, confirmed that a range of copy numbers for the various constructs existed. As few as two copies per diploid genome of Tropoprom-3 were integrated in one founder line and 52 copies of Tropoprom-2 were integrated in another transgenic founder (Table 1).

To assess the transcriptional expression of the integrated transgenes through the appearance of mature rat tropoelastin mRNA in transgenic mouse RNA preparations, we initially determined the complete derived amino acid sequence of mouse tropoelastin from several overlapping RT-PCR products obtained from mouse tropoelastin mRNA (Wydner et al 1994). A comparison of the coding sequence from mouse and rat tropoelastin message allowed us to synthesize mouse- and rat-specific oligomers. Species-specific primer pairs were then used to confirm, by RT-PCR analysis, the presence of rat tropoelastin mRNA in several tissues from F_1 progeny of the original founder transgenic mice. Screening progeny from 25 of the founder lines (three founder transgenic mice containing Tropoprom-2 constructs did not produce any progeny) revealed that 32% of these original founder lines did not synthesize detectable levels of rat tropoelastin mRNA. All the other founder lines expressed varying

TABLE 1 Relative levels of expression of rat and mouse tropoelastin mRNA in transgenic mouse tissue

Construct	*Founder line number*	*Relative levels of rat tropoelastin mRNA*					*Transgene copy number*
		Skin	*Lung*	*Kidney*	*Liver*	*Aorta*	
Tropoprom-1	508	809	26	189	812	ND	ND
	507	325	28	66	508	ND	25
Tropoprom-2	510	32	11	269	1080	5	2
	510*	ND	84	1111	1250	ND	2
	513	400	13	433	1101	6	52
	516	66	10	88	633	ND	19
Tropoprom-3	525	95	23	633	1320	7	ND
Tropoprom-4	539	46	5	22	177	4	3
	539*	115	35	304	1036	ND	3
	540	196	20	768	767	20	ND

Total RNA was isolated from several tissues from progeny of eight founder lines of transgenic mice. Rat and mouse tropoelastin mRNA was quantitated by laser densitometric scanning of autoradiograms of radiolabelled PCR products synthesized as described in the legend to Fig. 2. Levels of endogenous mouse tropoelastin mRNA were set in each tissue at 100%. Values presented reflect steady-state levels of rat tropoelastin mRNA. We obtained all the results by using RNA isolated from neonatal animals (2–3 days old) except for the determinations using progeny from the founder lines marked with an asterisk: tissue was obtained from these animals at 5 weeks of age. ND, not done.

levels of a mature, correctly processed rat tropoelastin mRNA that, appropriately, lacked intron 35.

Levels of expression of transcriptionally active transgenes were assessed by the use of a quantitative RT-PCR assay in which relative steady-state levels of both mouse and rat tropoelastin mRNA were measured (Fig. 2). A set of oligonucleotide primers were designed that were complementary to a region within exon 9 and exon 17 that was identical in both mouse and rat tropoelastin mRNA. RT-PCR amplification using these primers resulted in the synthesis of a 510 bp product from mouse total RNA and an almost identically sized 516 bp product from rat total RNA. To identify these DNA fragments, we took advantage of some unique restriction sites. The 516 bp rat amplimer, for example contained a single *Apa*I site. Digestion with *Apa*I produced 156 bp and 360 bp fragments. The mouse amplimer lacked this restriction site. *Apa*I digestion of co-amplified RT-PCR products from both mouse and rat tropoelastin mRNA, therefore, would produce restriction fragments readily separable by polyacrylamide gel electrophoresis (Fig. 2). Radiolabelling these restriction fragments allowed us to quantitate the amount of each species-specific amplimer by densitometric scanning of autoradiograms.

Using this assay, we screened total RNA preparations from newborn progeny obtained from several founder transgenes and the results are summarized in Table 1.

It is clear from these integration and expression studies that the 3.2 kb promoter domain used in the construction of the rat tropoelastin minigene recombinants is, in many tissues, a strong promoter responsible for the synthesis of abundant levels of rat tropoelastin mRNA readily detectable by our RT-PCR assay. The regulation of transcription by this promoter domain is, however, complex; evaluation of tissue-specific expression of the rat minigenes indicated that appropriate expression varied according to the type of tissue. For example, levels of expression of the rat transgenes in skin was usually comparable to or exceeded that of the endogenous expression of the mouse gene. In lung and aorta, however, transgene expression levels were significantly lower than the endogenous gene. In mouse kidney and liver, rat tropoelastin mRNA levels far exceeded the expression of the endogenous gene. This varied expression of the rat minigene constructs in different tissues suggests that different *cis*-acting elements within or flanking the tropoelastin gene are responsible for tissue-specific regulation of transcription. The *cis*-acting elements responsible for expression of the tropoelastin gene in skin seem to be represented within the 3.2 kb promoter domain present in the rat transgenic constructs. In contrast, the *cis*-acting DNA sequences necessary for appropriate expression in lung and aortic tissue are not present in the transgenic construct. In particular, the *cis*-acting elements required for inhibition of expression in non-elastogenic tissues such as kidney and liver are absent from

opoelastin mRNA that, appropria-

active transgenes were assessed by
n which relative steady-state levels
\ were measured (Fig. 2). A set of
t were complementary to a region
dentical in both mouse and rat
on using these primers resulted in
nouse total RNA and an almost
otal RNA. To identify these DNA
iique restriction sites. The 516 bp
gle *Apa*I site. Digestion with *Apa*I
The mouse amplimer lacked this
fied RT-PCR products from both
refore, would produce restriction
mide gel electrophoresis (Fig. 2).
s allowed us to quantitate the
y densitometric scanning of auto-

NA preparations from newborn
transgenes and the results are

ression studies that the 3.2 kb pro-
of the rat tropoelastin minigene
ng promoter responsible for the
in mRNA readily detectable by our
on by this promoter domain is, how-
expression of the rat minigenes
d according to the type of tissue.
at transgenes in skin was usually
logenous expression of the mouse
expression levels were significantly
kidney and liver, rat tropoelastin
the endogenous gene. This varied
in different tissues suggests that
nking the tropoelastin gene are
of transcription. The *cis*-acting
tropoelastin gene in skin seem to
omain present in the rat transgenic
sequences necessary for appropri-
re not present in the transgenic
ments required for inhibition of
kidney and liver are absent from

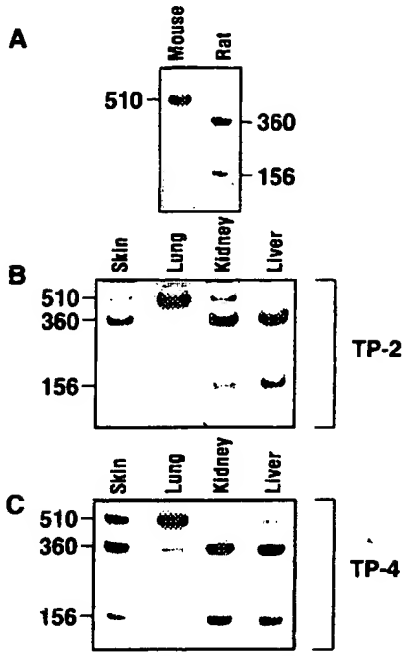


FIG. 2. A RT-PCR assay for the relative quantitation of rat and mouse tropoelastin mRNA in elastic tissue from transgenic mice. Oligonucleotide primers were designed that were complementary to a cDNA sequence encoded by exon 9 (MR9) and exon 17 (MR17) in the rat tropoelastin gene that was identical to coding sequence within the analogous domains in mouse tropoelastin mRNA. (A) Following reverse transcription of tropoelastin mRNA using Moloney murine leukaemia virus reverse transcriptase and random hexamer primers, polymerase chain amplification using MR9 and MR17 primers resulted in a 516 bp fragment from rat tropoelastin mRNA and a 510 bp fragment from mouse tropoelastin mRNA. *Apa*I digestion of PCR products generated 360 bp and 156 bp fragments from amplimers derived from rat tropoelastin mRNA. In contrast, the mouse-derived PCR product did not contain an *Apa*I site. PCR products were radiolabelled by including [^{32}Pd]CTP in the amplification reaction. Radiolabelled DNA fragments were size-separated by electrophoresis through 8% polyacrylamide. Polyacrylamide gels were dried and exposed to X-ray film. The sizes of restriction fragments (in bp) were calculated from molecular weight DNA markers (1 kbp DNA ladder) run in parallel. Panel (A) illustrates the recovery of PCR products, following *Apa*I digestion, obtained from RNA isolated from non-transgenic neonatal mouse and rat skin. Panels (B) and (C) illustrate the recovery of *Apa*I restriction fragments obtained from PCR amplimers prepared from RNA isolated from tissue samples of neonatal Tropoprom-2 (TP-2) and Tropoprom-4 (TP-4) transgenic mice.

the transgenic recombinants. The low levels of endogenous expression in these tissues are most likely to originate from the vasculature.

Another striking observation, clearly evident from these expression studies, is that the regions of the tropoelastin gene necessary for the developmental

regulation of expression are completely absent from the rat minigene constructs (Table 1). While endogenous levels of mouse tropoelastin mRNA were shown to decline in total RNA isolated from older transgenic progeny (five-week-old mice) rat tropoelastin mRNA continued to be expressed at levels comparable to those detected in neonatal tissue.

Incorporation of rat tropoelastin into elastic fibres in transgenic mice

It is clear from the analysis of rat tropoelastin mRNA levels described earlier that all four minigene constructs are transcriptionally active in the majority of the founder transgenic lines created. As an additional prerequisite to an evaluation of the effect of expression of a rat tropoelastin gene mutation on elastic fibre formation in transgenic mice, we also analysed the incorporation of rat tropoelastin into insoluble elastin isolated from several transgenic mouse elastic tissues.

We could not distinguish between mouse and rat elastin or tropoelastin using an immunohistochemical approach because antibodies specific to either rat or mouse tropoelastin are not available. To distinguish therefore between rat and mouse tropoelastin within a preparation of insoluble elastin, we took advantage of the observation by Sandberg and co-workers that high-performance liquid chromatography (HPLC) profiles of thermolytic digests of isolated insoluble elastin can be species specific (Sandberg et al 1990). These investigators had previously shown clear differences in HPLC profiles of thermolytic digests of insoluble elastin isolated from sheep and rat elastic tissue (Sandberg et al 1990). More recently, these investigators also demonstrated profile differences between preparations of insoluble elastin isolated from mouse and rat elastic tissue (L. B. Sandberg, P. J. Roos, unpublished observations). These observations immediately suggested a means to identify peptides from rat and mouse tropoelastin isolated from the same tissue.

In collaboration with Dr Sandberg, we demonstrated that differences in HPLC profiles between thermolytic digests obtained from rat and mouse insoluble elastin arose principally through differences in the frequency of hydrophobic peptide repeats within elastin from the two species (Fig. 3). For example, from a comparison of the complete amino acid sequence of mouse and rat tropoelastin, it is apparent that the hexapeptide VGGVPG is repeated six times in mouse tropoelastin and only four times in rat tropoelastin (Wydner et al 1994). The largest peak in the HPLC profile prepared from mouse elastin is obtained after 37 minutes of elution (we have referred to this peak as P37). Protein sequence analysis of P37 revealed that it contained six copies of VGGVPG. Sequence analysis of a thermolytic peptide recovered from rat elastin after 37 minutes of elution from the HPLC column revealed, predictably, only three copies of the same hexapeptide repeat. The size of the P37 peak obtained from rat elastin is, as expected, proportionately smaller than the P37 peak in the HPLC profile obtained from mouse elastin.

y absent from the rat minigene
evels of mouse tropoelastin mRNA
ated from older transgenic progeny
A continued to be expressed at levels
tissue.

c fibres in transgenic mice

n mRNA levels described earlier that
ionally active in the majority of the
ional prerequisite to an evaluation of
gene mutation on elastic fibre forma-
he incorporation of rat tropoelastin
transgenic mouse elastic tissues.
and rat elastin or tropoelastin using
e antibodies specific to either rat or
istinguish therefore between rat and
n of insoluble elastin, we took
lberg and co-workers that high-
LC) profiles of thermolytic digests
ies specific (Sandberg et al 1990).
clear differences in HPLC profiles
isolated from sheep and rat elastic
ly, these investigators also demon-
ations of insoluble elastin isolated
Sandberg, P. J. Roos, unpublished
ately suggested a means to identify
isolated from the same tissue.
e demonstrated that differences in
sts obtained from rat and mouse
h differences in the frequency of
from the two species (Fig. 3). For
lete amino acid sequence of mouse
hexapeptide VGGVPG is repeated
ır times in rat tropoelastin (Wydner
profile prepared from mouse elastin
have referred to this peak as P37).
d that it contained six copies of
olytic peptide recovered from rat
m the HPLC column revealed,
hexapeptide repeat. The size of the
ected, proportionately smaller than
l from mouse elastin.

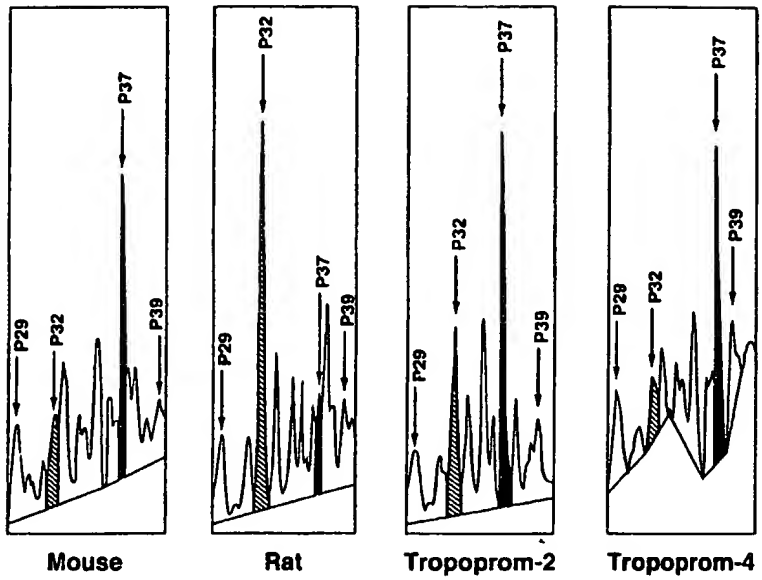


FIG. 3. HPLC profiles of thermolytic peptides obtained from insoluble elastin isolated from rat, mouse and transgenic mouse skin. HPLC of peptides isolated from insoluble elastin following digestion with thermolysin was carried out as previously described (Sandberg et al 1990). The largest peak obtained from rat elastin was evident after 32 min elution off the HPLC column (P32). The largest peak obtained from mouse elastin was evident after 37 min elution (P37). These peaks are indicated on the chromatograms together with the elutions times of several other peaks (in min). The various chromatograms were obtained using neonatal skin tissue from various sources, as indicated.

Similarly, the largest peak in an HPLC profile of a thermolytic digest of rat elastin was recovered after 32 min of elution (a peak we have called P32). Sequence analysis of this peptide revealed the presence of the tetrapeptides ALPG and AVPG. ALPG is repeated four times and AVPG is present at three copies in rat tropoelastin. A P32 peak is present in the HPLC profile obtained from mouse elastin; the size of the P32 peak correlates exactly with the representation of ALPG and AVPG as single copy peptides in mouse elastin.

The size of both the P37 and P32 peaks are clearly an indication of the phylogenetic origins of the peptides represented by these peaks (Fig. 3). Therefore, we used the P32:P37 ratio to define the difference between rat and mouse elastin. It is apparent from Table 2 that a P32:P37 ratio of 1.414 is typical for elastin isolated from rat skin. In contrast, a P32:P37 ratio of 0.598 was obtained from mouse skin elastin.

TABLE 2 P32:P37 ratios obtained from HPLC profiles of thermolytic digests of rat, mouse and transgenic mouse skin

Tissue source	*P32: P37 ratio*
Rat	1.085
Rat	1.285
Rat	1.872
Normal mouse	0.635
Normal mouse	0.560
Tropoprom-1 (#507)	1.567
Tropoprom-2 (#513)	1.082
Tropoprom-2 (#518)	1.067
Tropoprom-3 (#537)	2.151
Tropoprom-4 (#539)	0.292

P32 and P37 peaks were isolated as fractions from the HPLC column. An amino acid composition was then determined for each peak. Total peptide concentration in each peak was determined from this compositional analysis (in nanomoles). The amount of peptide recovered in each peak was then normalized to the total amount of peptide applied to the HPLC column. These normalized values (nanomoles/mg) were then compared to provide the ratios reported in this table. Determinations from skin obtained from three different neonatal rats are presented. The average of these ratios is 1.414. Two determinations are presented from non-transgenic neonatal mouse skin. The average of these ratios is 0.598.

P32:P37 ratios were then calculated from HPLC profiles obtained from thermolytic digests of insoluble elastin obtained from the skin of age-matched progeny of several founder transgenic mice. The calculated ratios ranged from 1.067 to 2.151. This peak ratio was significantly different to the ratio recovered from non-transgenic mouse skin and clearly demonstrated the contribution of rat peptides to the increased size of the P32 peak and concomitant increase in the P32:P37 ratio. Ratios recovered from some of the transgenic mice were intermediate between ratios obtained from normal rats and mice, and reflected the presence of chimeric elastin, composed of a mixture of rat and mouse tropoelastin. In skin from other transgenic mice, the P32:P37 ratio is approximately the same as the peak ratio recovered from normal rat skin. These skin samples were obtained from progeny in which we had previously shown high levels of expression of the transgenic constructs. The tetrapeptide repeats present in the P32 peak are three and four times more abundant in rat elastin than mouse elastin. Overexpression of a rat transgene, therefore, will readily mask any contribution of these peptides synthesized from the mouse tropoelastin gene.

ed from HPLC profiles of
d transgenic mouse skin

P32 : P37 ratio
1.085
1.285
1.872
0.635
0.560
1.567
1.082
1.067
2.151
0.292

tions from the HPLC column.
termined for each peak. Total
was determined from this
s). The amount of peptide
alized to the total amount of
n. These normalized values
provide the ratios reported in
btained from three different
of these ratios is 1.414. Two
n-transgenic neonatal mouse
8.

om HPLC profiles obtained from
ined from the skin of age-matched
. The calculated ratios ranged from
ntly different to the ratio recovered
y demonstrated the contribution of
peak and concomitant increase in
some of the transgenic mice were
normal rats and mice, and reflected
d of a mixture of rat and mouse
nic mice, the P32:P37 ratio is
recovered from normal rat skin.
ogeny in which we had previously
sgenic constructs. The tetrapeptide
d four times more abundant in rat
of a rat transgene, therefore, will
tides synthesized from the mouse

The P32:P37 ratio determined from skin isolated from transgenic mice containing transcriptionally active forms of the minigene constructs Tropoprom 1–3 were all significantly different from non-transgenic mouse ratios. Clearly, the rat tropoelastin mRNA we had observed earlier in transgenic mouse tissue was responsible for the synthesis of tropoelastin that was not only secreted but also incorporated into elastic fibre.

The P32:P37 ratio recovered from skin obtained from transgenic mice expressing the Tropoprom-4 construct is strikingly different to any ratio previously seen in skin samples from either transgenic or non-transgenic mice (Fig. 3 and Table 2). The Tropoprom-4 construct lacks exons 19–31. The repeating tetrapeptides ALPG and AVPG are encoded by exon 22 in the rat tropoelastin gene (Pierce et al 1990). Consequently, expression of the truncated rat tropoelastin gene present in the Tropoprom-4 recombinant should not contribute thermolytic peptides to the P32 peak. Similarly, a single copy of the hexapeptide VGGVPG, encoded within exon 24 of the rat tropoelastin gene, should also not be represented in the truncated tropoelastin synthesized by the Tropoprom-4 construct. However, exon 7 of the rat gene encodes two copies of VGGVPG and these hexapeptides will be represented in the aberrant protein synthesized from Tropoprom-4. The presence of a truncated rat tropoelastin containing two copies of VGGVPG should contribute to an increased size of the P37 peak which already represents six copies of the hexapeptide contributed from the endogenous expression of the normal mouse tropoelastin gene. No change in the size of the P32 peak but an increased P37 peak as a consequence of Tropoprom-4 expression would result in a P32:P37 ratio that would be less than the ratio recovered from normal mouse skin elastin. This is precisely the result we obtained (Table 2). The P32:P37 ratio from transgenic mice containing the Tropoprom-4 construct demonstrated the presence of a truncated tropoelastin incorporated into elastin. The unusual ratio also confirmed the validity of our assay.

The phenotypic consequences of expression of the Tropoprom constructs in transgenic mice

The first indications of an abnormal phenotype were evident in the founder transgenic mice and progeny containing the Tropoprom construct (Tropoprom-2) that lacked exon 33. Of the 11 founder mice established with integrated Tropoprom-2 recombinants, no transgenic progeny could be established from four as a consequence of an unusually high incidence of maternal cannibalism, a well-known indicator of possible defects in newborn animals. In addition, from those progeny that were successfully propagated from founder mice containing Tropoprom-2 constructs, no homozygous animals were ever obtained from hemizygous brother–sister matings, once again as a result of maternal cannibalism.

While difficulties in breeding are very suggestive of defects in newborn mice, the first real evidence demonstrating that such defects exist was found in the progeny from two separate founder lines expressing Tropoprom-2. Several of the progeny from these two founder transgenics died at 5–7 weeks of age as a result of respiratory distress. Autopsy of multiple progeny revealed abnormally small and non-compliant lungs. Histological sections prepared from pulmonary tissue from affected transgenic mice and non-transgenic litter mates revealed marked enlargement of alveoli and extensive septal damage (Fig. 4). These pulmonary changes were typical of pulmonary alterations associated with the development of emphysema. These emphysematous changes occurred in the progeny from separate founder transgenic mice actively transcribing the Tropoprom-2 construct. No such lung defects were present in transgenic progeny containing transcriptionally active Tropoprom-1 recombinants, constructs containing the complete sequence for rat tropoelastin. Emphysema did not develop, therefore, in the Tropoprom-2-containing progeny as a consequence of the incorporation of rat tropoelastin into lung elastin nor did a pulmonary defect arise as a result of the disruption of a mouse gene at a site of integration of a Tropoprom-2 recombinant into mouse genomic DNA. Many of our transgenic (F_1) mice, therefore, hemizygous for the Tropoprom-2 recombinant, develop an emphysematous phenotype as a result of the aberrant overexpression of a tropoelastin isoform lacking the hydrophobic peptide encoded by exon 33.

We observed a second, completely different, phenotype in transgenic mice expressing high levels of the Tropoprom-4 construct. This recombinant lacks exons 19–31 of the rat tropoelastin gene. We successfully established three founder lines in which Tropoprom-4 had been integrated into the mouse genome. One of these founder lines did not express the transgene but two separate founder mice expressed this truncated minigene construct. Progeny from founder mice expressing Tropoprom-4 were abnormally small. Hemizygous F_2 progeny from F_1 females continued to express the minigene. Litter sizes were small in number, transgenic offspring were small in size and the mice died within 2–3 days of birth. The F_1 progeny and founder mice died between 7–9 months of age. Autopsy revealed massive internal haemorrhage. The lungs, however, appeared normal. Histopathological evaluation revealed a very abnormal ascending aorta (Fig. 4), characterized by hypoplastic and disorganized medial elastic laminae. In some histopathological sections in particular, a complete loss of medial elastic fibres was noticed together with a disrupted adventitia and even evidence for complete rupture of the aortic wall. This histological evidence is entirely consistent with the development of an ascending aortic aneurysm.

In contrast to the dramatic and contrasting phenotypes observed in some of the transgenic mice containing Tropoprom-2 and Tropoprom-4 recombinants, founder transgenes containing Tropoprom-1 and Tropoprom-3 constructs did

suggestive of defects in newborn
that such defects exist was found in
es expressing Tropoprom-2. Several
ansgenics died at 5–7 weeks of age
psy of multiple progeny revealed
igs. Histological sections prepared
genic mice and non-transgenic litter
lveoli and extensive septal damage
typical of pulmonary alterations
nphysema. These emphysematous
separate founder transgenic mice
nstruct. No such lung defects were
anscriptionally active Tropoprom-1
complete sequence for rat tropo-
ore, in the Tropoprom-2-containing
ation of rat tropoelastin into lung
as a result of the disruption of a
poprom-2 recombinant into mouse
$^{2}_{1}$) mice, therefore, hemizygous for
n emphysematous phenotype as a
a tropoelastin isoform lacking the

rent, phenotype in transgenic mice
m-4 construct. This recombinant
gene. We successfully established
had been integrated into the mouse
not express the transgene but two
cated minigene construct. Progeny
1-4 were abnormally small. Hemi-
ued to express the minigene. Litter
ring were small in size and the mice
ny and founder mice died between
assive internal haemorrhage. The
athological evaluation revealed a
characterized by hypoplastic and
ome histopathological sections in
fibres was noticed together with a
complete rupture of the aortic wall.
stent with the development of an

ng phenotypes observed in some of
2 and Tropoprom-4 recombinants,
1 and Tropoprom-3 constructs did

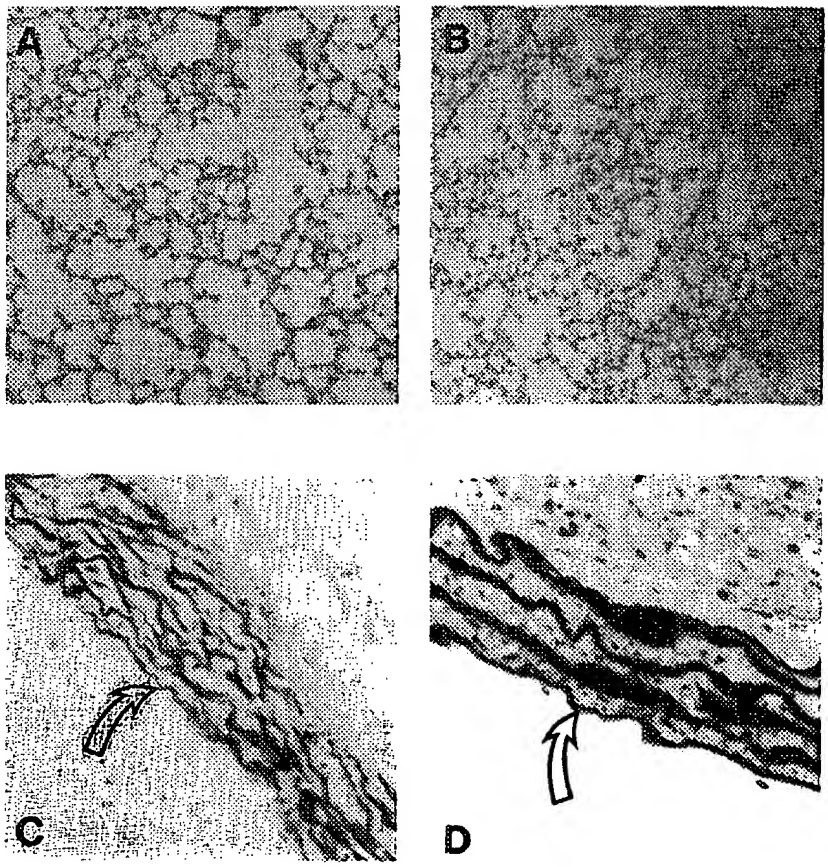


FIG. 4. Histopathologic sections of lung and aortic tissue obtained from transgenic mice containing Tropoprom-2 and Tropoprom-4 recombinants. Tropoprom-2 (−exon 33). (A) Haematoxylin and eosin-stained lung sections from a transgenic mouse expressing Tropoprom-2 and (B) a non-transgenic litter mate. Original magnification in both sections is × 100. Tropoprom-4 (−exons 19–31). (C) Verhoeff van Giesen elastin fibre stain of a section of the ascending aorta from a transgenic mouse expressing high levels of Tropoprom-4 and (D) an age-matched transgenic mouse expressing Tropoprom-1 (+exons 1–36). The arrows indicate the position of the internal elastic lamina. Original magnification in both sections is × 133.

not reveal any elastic tissue phenotype or any abnormalities in breeding patterns or behaviour. Transcriptionally active Tropoprom-1 (encoding the complete sequence of rat tropoelastin) and Tropoprom-3 (a construct lacking exon 12–15) minigenes were successfully established in a total of eight separate

founder lines. Several of these transgenes were shown to express some of the highest levels of rat tropoelastin mRNA of any of the transgenes analysed. Large litters, however, were common and maternal cannibalism was not encountered. Moreover, histopathological evaluation of lung and aortic tissue from both newborn and neonatal transgenes revealed normal elastic fibre morphology.

Conclusions

The synthesis of the complete sequence of rat tropoelastin in the progeny of multiple founder transgenic mice and the assembly of a mouse–rat elastin chimaera does not seem to influence elastic fibre assembly or elastic tissue function. Similarly, overexpression of the rat tropoelastin isoform lacking exons 13–15 does not result in any obvious aberrant phenotype. It would seem therefore that, within the elastic tissues analysed (aorta, lung and skin), this particular isoform of tropoelastin is unnecessary, during mouse development, for normal elastic fibre assembly and function.

In contrast, overexpression of the rat tropoelastin isoform lacking the domain encoded by exon 33 results in a relatively early-onset emphysematous phenotype. Emphysema is thought to be a disease caused by the elastase-catalysed degradation of pulmonary elastic fibres, leading to the destruction of alveolar septa, consequent alveolar enlargement and eventual loss of pulmonary function (Snider et al 1986, Janoff 1985). The results we have obtained with our Tropoprom-2 transgenic mice are not inconsistent with this hypothesis. Overexpression of the tropoelastin isoform lacking the domain encoded by exon 33 may result in the assembly of a structurally compromised elastic fibre, more susceptible to degradation either through the direct action of elastase or indirectly through mechanical stress, leading to alveolar wall damage and degradation of damaged elastic fibres by elastases.

The Tropoprom-4 construct resulted in the incorporation of a truncated tropoelastin into insoluble elastin in several elastic tissues in transgenic mice. The presence of this aberrant elastin in the ascending aorta produced a severe aneurysmal phenotype.

Recently, studies by Keating and co-workers (Curran et al 1993, Ewart et al 1994) have shown that large deletions on human chromosome 7q, involving the loss of part of the tropoelastin gene, are associated with the vascular disorder SVAS. Specifically, in two separate and unrelated patients, these investigators have shown the presence of a large deletion (Ewart et al 1994) and a balanced translocation (Curran et al 1993), both of which involved a breakpoint located at the 3′ end of the tropoelastin gene. There is a clear association between the appearance of these mutations and the SVAS phenotype. Linkage studies moreover have established that a mutation causing SVAS resides in the locus on the long arm of chromosome 7 that contains the tropoelastin gene (Ewart et al 1993a). It is not clear, however, whether the disruption of the tropoelastin gene

s were shown to express some of the
any of the transgenes analysed. Large
nal cannibalism was not encountered.
of lung and aortic tissue from both
l normal elastic fibre morphology.

of rat tropoelastin in the progeny of
ne assembly of a mouse–rat elastin
stic fibre assembly or elastic tissue
he rat tropoelastin isoform lacking
s aberrant phenotype. It would seem
nalysed (aorta, lung and skin), this
cessary, during mouse development,
ction.
t tropoelastin isoform lacking the
elatively early-onset emphysematous
e a disease caused by the elastase-
c fibres, leading to the destruction of
nlargement and eventual loss of
Janoff 1985). The results we have
ic mice are not inconsistent with this
elastin isoform lacking the domain
mbly of a structurally compromised
on either through the direct action of
al stress, leading to alveolar wall
tic fibres by elastases.
n the incorporation of a truncated
al elastic tissues in transgenic mice.
e ascending aorta produced a severe

rkers (Curran et al 1993, Ewart et al
uman chromosome 7q, involving the
ssociated with the vascular disorder
nrelated patients, these investigators
n (Ewart et al 1994) and a balanced
hich involved a breakpoint located at
: is a clear association between the
SVAS phenotype. Linkage studies
causing SVAS resides in the locus on
ns the tropoelastin gene (Ewart et al
e disruption of the tropoelastin gene

per se actually causes the SVAS phenotype. The large deletions observed in SVAS patients and in patients with a related disorder, Williams syndrome, also involve DNA flanking the tropoelastin gene (Ewart et al 1993b). Disruption of a gene in close proximity to the tropoelastin gene could actually be responsible therefore for SVAS and/or Williams syndrome.

The deletions affecting the 3′ end of the tropoelastin gene in some SVAS patients may result in the creation of a null allele. The mutation could also result in the synthesis of a truncated tropoelastin that, if incorporated into insoluble elastin, may result in the assembly of aberrant elastic fibres. Support for this latter view may be forthcoming from the curious observation that deletion of exons 19–31 in our Tropoprom-4 mice results in an ascending aortic abnormality with no other obvious elastic tissue dysfunction. It was not possible to determine whether these transgenic mice developed SVAS; the histopathology of elastic fibres in the ascending aorta is remarkably similar, however, to the disorganized elastic fibre morphology typical of SVAS (O'Connor et al 1985). Aneurysms, moreover, have been reported as a clinical complication of SVAS (Beitzke et al 1986). An exciting speculation therefore would be that the aberrant transcripts from our in-frame, intragenic deletion of exons 19–31 in the rat tropoelastin gene, that will not disrupt any other gene in the vicinity of the endogenous mouse tropoelastin gene, result in a severe form of SVAS that leads to aortic rupture. Should SVAS indeed be directly due to a disruption of elastic fibre assembly, through the synthesis of aberrant protein rather than a dosage effect as a consequence of a null allele, then it would seem very likely that the non-vascular components of Williams syndrome, particularly the neurodegenerative features of this disorder, may be due to disruption of gene(s) proximal to the tropoelastin gene on the long arm of human chromosome 7.

Methods

The details of the methods summarized in this manuscript have been fully described in several recent publications (Wydner et al 1994, Sechler et al 1995b, Sechler 1994, Alatawi 1994).

Acknowledgements

This work was supported by NIH grants [HL 37438], [HL 42798] and [HL 39869]. We gratefully acknowledge the assistance of DNX (Princeton, NJ) in preparing the founder transgenic mice and Gary Benson for typing the manuscript.

References

Alatawi A 1994 Characterization of the complete structure of the rat tropoelastin gene. PhD thesis, Rutgers University, NJ, USA

Beitzke A, Becker H, Rigler B, Stein JI, Suppan C 1986 Development of aortic aneurysms in familial supravalvular aortic stenosis. Pediatr Cardiol 6:227–229

Boyd CD, Christiano AM, Pierce RA, Stolle CA, Deak SB 1991 Mammalian tropoelastin: multiple domains of the protein define an evolutionarily divergent amino acid sequence. Matrix 11:235–241

Curran ME, Atkinson DL, Ewart AK, Morris CA, Leppert MF, Keating MT 1993 The elastin gene is disrupted by a translocation associated with supravalvular aortic stenosis. Cell 73:159–168

Deyl Z, Horakova M, Macek K 1985 Changes in elastin composition in aorta of spontaneously hypertensive (SHR) rats. Biochem Biophys Res Commun 129:179–186

Dietz HC, Cutting GR, Pyeritz RE et al 1991 Marfan syndrome caused by a recurrent *de novo* missense mutation in the fibrillin gene. Nature 352:337–339

Ewart AK, Morris CA, Ensing GJ et al 1993a A human vascular disorder, supravalvular aortic stenosis, maps to chromosome 7. Proc Natl Acad Sci USA 90:3226–3230

Ewart AK, Morris CA, Atkinson D et al 1993b Hemizygosity at the elastin locus in a developmental disorder, Williams syndrome. Nature Genet 5:11–16

Ewart AK, Jin W, Atkinson D, Morris CA, Keating MT 1994 Supravalvular aortic stenosis associated with a deletion disrupting the elastin gene. J Clin Invest 93: 1071–1077

Gibson MA, Sandberg LB, Grosso LE, Cleary EG 1991 Complementary DNA cloning establishes microfibril-associated glycoprotein (MAGP) to be a discrete component of the elastin-associated microfibrils. J Biol Chem 266:7596–7601

Glaubitz LC, Bowen JH, Cox EB, McCarty KS 1984 Elastosis in human breast cancer. Arch Pathol Lab Med 108:27–30

Heim RA, Pierce RA, Deak SB, Riley DJ, Boyd CD, Stolle CA 1991 Alternative splicing of rat tropoelastin pre-mRNA is developmentally regulated. Matrix 11: 359–366

Hollister DN, Godfrey M, Sakai LY, Pyeritz RE 1990 Immunohistologic abnormalities of the microfibrillar-fiber system in the Marfan syndrome. N Engl J Med 323:152–159

Jacenko O, LuValle PA, Olsen BR 1993 Spondylometaphyseal dysplasia in mice carrying a dominant negative mutation in a matrix protein specific for cartilage-to-bone transition. Nature 365:56–61

Jaenisch R 1988 Transgenic animals. Science 240:1468–1474

Janoff A 1985 Elastases and emphysema: current assessment of the protease–antiprotease hypothesis. Am Rev Respir Dis 132:417–433

Indik Z, Yeh H, Ornstein-Goldstein N, Rosenbloom J 1990 Structure of the elastin gene and alternative splicing of elastin mRNA. In: Sandell LJ, Boyd CD (eds) Extracellular matrix genes. Academic Press, San Diego, CA, p 221–250

Iredale RB, Eccleston-Joyner CA, Rucker RB, Gray SD 1989 Ontogenic development of the elastic component of the aortic wall in spontaneously hypertensive rats. Clin Exp Hypertens Part A Theory Pract 11:173–187

Kagan HM 1986 Characterization and regulation of lysyl oxidase. In: Mecham RP (ed) Regulation of matrix accumulation. Academic Press, San Diego, CA, p 321–398

Kramsch DM, Hollander W 1973 The interaction of serum and arterial lipoproteins with elastin of the arterial intima and its role in the lipid accumulation in atherosclerotic plaques. J Clin Invest 52:236–247

Lee G, Godfrey M, Vitale E et al 1991 Linkage of Marfan syndrome and a phenotypically related disorder to two different fibrillin genes. Nature 352:330–334

Mecham RP, Heuser JE 1991 The elastic fiber. In: Hay ED (ed) The cell biology of the extracellular matrix, 2nd edn. Plenum Press, New York, p 79–110

Mecham RP, Whitehouse LA, Wrenn DS et al 1987 Smooth muscle-mediated connective tissue remodeling in pulmonary hypertension. Science 237:423–426

Nelder K 1988 Pseudoxanthoma elasticum. Clin Dermatol 6:1–159

olle CA, Deak SB 1991 Mammalian
tein define an evolutionarily divergent

CA, Leppert MF, Keating MT 1993 The
n associated with supravalvular aortic

iges in elastin composition in aorta of
hem Biophys Res Commun 129:179–186
Marfan syndrome caused by a recurrent
ne. Nature 352:337–339
human vascular disorder, supravalvular
Natl Acad Sci USA 90:3226–3230
b Hemizygosity at the elastin locus in a
. Nature Genet 5:11–16
Keating MT 1994 Supravalvular aortic
ing the elastin gene. J Clin Invest 93:

EG 1991 Complementary DNA cloning
1 (MAGP) to be a discrete component of
m 266:7596–7601
1984 Elastosis in human breast cancer.

Boyd CD, Stolle CA 1991 Alternative
developmentally regulated. Matrix 11:

E 1990 Immunohistologic abnormalities
n syndrome. N Engl J Med 323:152–159
ondylometaphyseal dysplasia in mice
matrix protein specific for cartilage-to-

40:1468–1474
current assessment of the protease–
132:417–433
oom J 1990 Structure of the elastin gene
A. In: Sandell LJ, Boyd CD (eds)
San Diego, CA, p 221–250
Gray SD 1989 Ontogenic development
1 spontaneously hypertensive rats. Clin
187
n of lysyl oxidase. In: Mecham RP (ed)
c Press, San Diego, CA, p 321–398
ion of serum and arterial lipoproteins
le in the lipid accumulation in athero-

inkage of Marfan syndrome and a
nt fibrillin genes. Nature 352:330–334
n: Hay ED (ed) The cell biology of the
New York, p 79–110
87 Smooth muscle-mediated connective
Science 237:423–426
1 Dermatol 6:1–159

O'Connor W, Davis J, Geissler R, Cottrill C, Noonan J, Todd E 1985 Supravalvular aortic stenosis: clinical and pathologic observations in six patients. Arch Pathol Lab Med 109:179–185

Olsen DR, Fazio MJ, Shamban AT, Rosenbloom J, Uitto J 1988 Cutis laxa: reduced elastin gene expression in skin fibroblast cultures as determined by hybridizations with a homologous cDNA and an exon 1-specific oligonucleotide. J Biol Chem 263:6465–6467

Palmiter RD, Brinster RL 1986 Germ-line transformation of mice. Annu Rev Genet 20:465–499

Parks WC, Deak SB 1990 Tropoelastin heterogeneity: implications for protein function and disease. Am J Respir Cell Mol Biol 2:399–406

Pierce RA, Alatawi A, Deak SB, Boyd CD 1992 Elements of the rat tropoelastin gene associated with alternative splicing. Genomics 12:651–658

Pierce RA, Deak SB, Stolle CA, Boyd CD 1990 Heterogeneity of rat tropoelastin mRNA revealed by cDNA cloning. Biochemistry 29:9677–9683

Rosenbloom J 1984 Elastin: relation of protein and gene structure to disease. Lab Invest 51:605–623

Sandberg LB, Roos PJ, Pollman MJ, Hodgkin DD, Blankenship JW 1990 Quantitation of elastin in tissues and culture: problems related to the accurate measurement of small amounts of elastin with special emphasis on the rat. Connect Tissue Res 25:139–148

Sechler JL 1994 Elastin gene mutations in transgenic mice. PhD thesis, Rutgers University, NJ, USA

Sechler JL, Cardone JC, Friedmann J et al 1995a Altered tropoelastin gene expression is associated with the development of hypertension in the spontaneously hypertensive (SHR) rat. Connect Tissue Res, in press

Sechler JL, Sandberg LB, Roos PJ, Amenta PS, Riley DJ, Boyd CD 1995b Overexpression of an alternative splice variant of tropoelastin in transgenic mice results in lung abnormalities resembling pulmonary emphysema. J Biol Chem, submitted

Snider GL, Lucey EC, Stone PJ 1986 Animal models of emphysema. Am Rev Respir Dis 133:149–169

Uitto J, Shamban A 1987 Heritable skin diseases with molecular defects in collagen or elastin. Clin Dermatol 5:63–84

Uitto J, Fazio MJ, Olsen DR 1989 Molecular mechanisms of cutaneous aging. J Am Acad Dermatol 21:614–622

Wydner KS, Sechler JL, Boyd CD, Passmore HC 1994 Use of an intron length polymorphism to localize the tropoelastin gene to mouse chromosome 5 in a region of linkage conservation with human chromosome 7. Genomics 23:125–131

DISCUSSION

Parks: You showed that transgenic mice expressing Tropoprom-2 had altered lung morphology (Fig. 4), yet your PCR data showed very little expression of Tropoprom-2 in the lung (Table 1). However, it was expressed at high levels in the skin. Do you know if the morphology of the elastic fibres in the skin of these transgenic animals is altered?

Boyd: We haven't looked yet.

Parks: How do you suppose that such a low level of Tropoprom-2 expression would alter lung morphology in these mice?

Boyd: We have speculated that there are two essential effects. One is that even low levels of expression of this particular isoform at an inappropriate time in lung development will result in the assembly of a fibre that can't cope with the mechanical forces that are required of it in the alveolar septum. Consequently, the elastic fibres rupture, the alveolar walls are disrupted and this leads to the larger spaces that are seen in the histopathology. This degenerative process is a characteristic of the emphysematous phenotype. Our other speculation is that this has nothing to do with mechanical stress, but instead fibres are assembled that are much more susceptible to proteolysis. As a result, there is rapid degradation of elastic fibres by elastase in the pulmonary tissue. This is the scenario we favour, because apart from the lung damage we see no other phenotype in these animals. It is possible that pulmonary tissue is more severely affected than any other tissue, independent of the very low level of Tropoprom-2 expression in pulmonary tissue, because that's where proteolytic susceptibility may be much more critical.

Parks: But in the micrographs you showed there was no overt inflammation, which makes me wonder where these potential proteases would be coming from. I noticed that there was a high level of expression of Tropoprom-2 mRNA in the kidney and liver, but was there any evidence of abnormal deposition of elastic fibres in these organs?

Boyd: If your *in situ* hybridizations had worked, we would know!

Parks: No, *in situ* hybridization is an assay of mRNA expression, not protein deposition. Your PCR results showed that there is significant expression of the transgene in kidney and the liver, so I was wondering if these tissues have the capacity to deposit elastic fibres outside of their vasculature?

Boyd: We haven't started a systematic analysis of the various tissues in which we have elicited expression—this obviously needs to be done.

Starcher: I was also surprised that even though there was very little expression of Tropoprom-2, you still found an effect in the lung. But I caution you about calling the lung damage you saw 'emphysema'. You really have to show that there's actual degradation of the alveolar walls to be able to do this, and I didn't notice this in your micrographs. In emphysematous lungs we usually see balls of retracted elastin where the alveoli are broken. The degree of abnormal lung development in your micrographs was really very slight, when you look at the changes in the mean linear intercepts. We and other people have done experiments where we pour elastases into lungs and create emphysema, and even though there is not much of the lung structure left in these animals, they still manage to breathe and they survive. I've seen lungs where you can hardly find a piece of lung without a hole in it, and the animals don't suffocate. I would have also anticipated that you would have seen more of a fibrotic effect than emphysema, because I did not see any inflammation. We've done experiments where we put lipopolysaccharide into the lungs and you get a continuous progression of neutrophils coming in the lungs. Over a

re two essential effects. One is that
ticular isoform at an inappropriate
: assembly of a fibre that can't cope
uired of it in the alveolar septum.
he alveolar walls are disrupted and
seen in the histopathology. This
the emphysematous phenotype. Our
; to do with mechanical stress, but
more susceptible to proteolysis. As a
fibres by elastase in the pulmonary
use apart from the lung damage we
: is possible that pulmonary tissue is
:e, independent of the very low level
ary tissue, because that's where
re critical.
ed there was no overt inflammation,
tential proteases would be coming
vel of expression of Tropoprom-2
s there any evidence of abnormal
?

worked, we would know!
ty of mRNA expression, not protein
there is significant expression of the
wondering if these tissues have the
their vasculature?
alysis of the various tissues in which
/ needs to be done.
ven though there was very little
l an effect in the lung. But I caution
w 'emphysema'. You really have to
alveolar walls to be able to do this,
iphs. In emphysematous lungs we
he alveoli are broken. The degree of
graphs was really very slight, when
ir intercepts. We and other people
elastases into lungs and create
much of the lung structure left in
: and they survive. I've seen lungs
ithout a hole in it, and the animals
:ed that you would have seen more
se I did not see any inflammation.
polysaccharide into the lungs and
ophils coming in the lungs. Over a

long period of time we see some eroding of the lung, but these animals live and you eventually get a fibrotic condition.

Boyd: We've been told by our pulmonary expert physician that the mean linear intercept differences we've seen are consistent with the differences that are often seen in human emphysematous conditions, particularly in early-onset emphysema (David Riley, personal communication). Remember, in order to do the mean linear intercept calculations we use animals a couple of weeks before they start dying of a breathing disorder (these are three-week-old animals; they start dying between five and seven weeks of age). At this stage, the development of the disease is still fairly mild.

Starcher: This is another problem. When you look at very young animals, the alveoli are still being formed. The alveolar walls are being formed at around Day 10 to 12, so you may not have reached maturity in the lung by three weeks. You may be seeing a problem in lung maturation rather than degradation.

Boyd: The Verhoeff van Giesen stains of the pulmonary tissue show lots of evidence for elastic fibres that have been disrupted in the alveolar wall. There's no doubt about that.

Sandberg: I just want to comment on the problem of the mean linear intercept that Barry Starcher raised. The definition of emphysema is really based on mean linear intercepts and it matters less what the histology looks like. Bad-looking lungs can still have normal mean linear intercepts and not have emphysema. What is important is that Charles Boyd was able to show in these very young animals that there was a statistically significant difference in mean linear intercepts. Enzymically induced lung damage involves an entirely different mechanism.

Uitto: There was obviously quite a lot of variation in the level of Tropoprom expression. Do you know whether this correlates with the transgene copy number and perhaps the site of integration? In fact, do you know where these transgenes are integrated?

Boyd: We haven't looked at the sites of integration, although we do know that the sites are different in the different founders. In certain tissues, such as skin, there seems to be a correlation between expression and copy number, but in other tissues there isn't. We have taken this to mean that there isn't any obvious correlation between copy number and expression.

Uitto: In the mouse line 513, I noted that there were 52 copies of the Tropoprom-2 transgene. Do you know if they all went into a single integration site?

Boyd: From the gene dosage results of Jan Sechler, it seems that there was indeed a single integration site for these 52 copies.

Davidson: Is the elastin content of the lungs in the Tropoprom-2 transgenic mice different from control litter mates?

Boyd: We don't know.

Davidson: How many of the different Tropoprom-2 lines express this phenotype?

Boyd: All those lines expressed high levels of the Tropoprom-2 construct.

Davidson: You have got a total of 28 lines using presumably the same *cis*-acting elements and the same 3′ UTR domain. Is there a consistent pattern of tissue-specific or developmentally related expression that relates to these regulatory portions of the molecule?

Boyd: There are a number of guidelines: it's hard to say whether there's an overall expression pattern that can be generalized. One certainly sees inappropriate expression of the transgenes in kidney and liver; this is the one thing that I think is consistent. The other is that the expression of all the founders continues into adult tissue. It is at times very hard to quantitate individual expression between various tissues, because there are enormous differences.

Davidson: Why hasn't anyone looked at whether or not the amount of elastin gene expression in SVAS patients is altered? Why hasn't this been more fully explored?

Boyd: This is because of the relatively small number of patients we've got. All of them are children, and parents are not necessarily that keen on providing skin fibroblast cultures. So at the present time, ourselves, Steve Thibodeau and Mark Keating have only been able to obtain informative genomic DNA samples.

Robert: I think your results with the transgenic mice showing different lung structures are very interesting and it is not necessary to look for a perfect analogy to any of the human situations. If a similar situation occurred in humans they would develop, for instance, more severe asthma or emphysema. This is a very good basis for medical speculations. Some of the diseases you mentioned in your introduction concerning anomalies in vascular development could also be connected. Why would the aorta get narrower if you have less elastin expression? It could instead get much larger and rupture. Modifications of elastin gene expression may lead to explanations concerning the mechanism of genetic regulation of the structure and morphology of blood vessels during development.

Mungai: Have you carried out any studies on the actual characteristics of the elastic fibres in those animals where the aorta ruptures?

Boyd: No; obviously that's a major priority. Presumably you are suggesting electron microscope and biochemical studies.

Mungai: Yes, and also biophysical studies: for instance, what is the elastic limit in relation to the normal physiological requirements?

Boyd: Those are terribly important experiments that ought to be done.

Ooyama: Most of the aneurysms we see are atherosclerotic. Do you think your tropoelastin knockout mice could be a model for atherosclerotic aneurysm?

it Tropoprom-2 lines express this

els of the Tropoprom-2 construct.
ines using presumably the same *cis*-
nain. Is there a consistent pattern of
d expression that relates to these

: it's hard to say whether there's an
e generalized. One certainly sees
s in kidney and liver; this is the one
er is that the expression of all the
is at times very hard to quantitate
ssues, because there are enormous

whether or not the amount of elastin
d? Why hasn't this been more fully

small number of patients we've got.
ot necessarily that keen on providing
ime, ourselves, Steve Thibodeau and
obtain informative genomic DNA

nsgenic mice showing different lung
iot necessary to look for a perfect
If a similar situation occurred in
more severe asthma or emphysema.
culations. Some of the diseases you
; anomalies in vascular development
aorta get narrower if you have less
h larger and rupture. Modifications
anations concerning the mechanism
norphology of blood vessels during

s on the actual characteristics of the
orta ruptures?
rity. Presumably you are suggesting
es.
ies: for instance, what is the elastic
al requirements?
riments that ought to be done.
: are atherosclerotic. Do you think
be a model for atherosclerotic

Boyd: We were surprised to find aortic rupture in the ascending aorta, because most aneurysms develop in the infrarenal aorta. We looked at the histopathology at seven months of age in the Tropoprom-4 construct and found no evidence for any disrupted elastic fibre morphology in that region of the aorta, which suggests that this may not be a model. But, of course, the seven-month-old mouse is not equivalent to a 60-year-old human—it may well be that we will have to leave these animals for longer. We've had a great deal of difficulty breeding these animals, because they die at seven months. The trick is to get a Tropoprom-4 mouse that expresses the construct at relatively low levels, see whether it will live beyond the seven-month limit we have experienced in the few constructs we've had so far, and then ask if histopathological abnormalities in the infrarenal aorta will develop.

Mariani: Could you describe your histochemical staining procedure?

Boyd: We used perfusion-fixed sections from three-week-old mice, and did both Verhoeff van Giesen and haemotoxylin and eosin staining. You can't really do quantitative morphology on a non-perfusion-fixed section. We were able to reproduce what we saw in the non-perfusion-fixed sections taken from an animal that died at five weeks of age. With the Verhoeff van Giesen stain we were able to see clear evidence for elastic fibre breakage and disruption, although at that very crude level we couldn't see any obvious morphological difference between the elastic fibres in the alveolar septum. We are going to have to do electron microscopy to be able to see those morphological differences.

Keeley: If you look at the bronchi in these animals, could their suffocation be due not to the lung changes but to bronchial changes?

Boyd: We haven't seen any elastic fibre differences in the bronchus.

Keeley: I'm interested in the Tropoprom-1 mouse, which is really just expressing the normal tropoelastin gene. This tropoelastin continues to be expressed in these animals throughout development, not turning off as it normally does, and, as well, it is produced in tissues not normally expressing elastin. This might result in some phenotypic changes in these animals, at least later in life. Did you see anything like that?

Boyd: We haven't looked, but what we are doing is to breed the transgenic Tropoprom-1 mouse with a hairless mouse, because the levels of expression in skin are very high. The progeny will be studied in a collaboration with Unilever, involving quantitative confocal microscopy on skin elastic fibres in order to address that very question.

Rosenbloom: I wonder if Bill Parks would like to comment on the continued expression of this kind of transgene, when his or Richard Pierce's data suggest that even if it were to be continually transcribed, the mRNA would probably be degraded.

Parks: Because he has shown continued expression of the transgene into the adult, Charles Boyd's results agree with ours. Our data show that the endogenous tropoelastin gene continues to be transcribed well into adulthood.

Boyd: Of course, our assay is for message, not pre-message.

Parks: That's fine: thus the elements in the pre-mRNA or the processed mRNA that control the post-transcriptional regulation of tropoelastin are not present in your minigenes. Our favourite hypothesis is that this mechanism is mediated by something in the 3′ UTR. Since you're seeing continued expression of minigene mRNA, which does include the 3′ UTR, I think we might have to modify our idea a bit and consider other parts of the gene, such as intron 1, which is a great candidate for a regulatory element and is not present in your minigenes.

Boyd: We've come to the same conclusion; that some intron sequence absent in the minigenes was obviously mediating a post-transcriptional control in pre-message availability which would then be consistent with the results you've got.

Parks: We think of elastin assembly as a process that requires numerous products and that there must be a precise, co-ordinate regulation of these products and their stoichometry. In your model, you're supplying more of the principal substrate, namely, tropoelastin. Do you think that more elastin will be made in these animals and how does that affect our thinking about the mechanisms of fibre assembly?

Boyd: Good quantitative biochemistry is going to be important to answer this. At a very gross level, there's no obvious thickening of the elastic fibres (as I showed you in that elastic fibre stain of the ascending aorta from the Tropoprom-1 construct; Fig. 4D). There's a huge increase in the total amount of tropoelastin available for elastic fibre assembly in those animals, but we don't see an obvious increase in the thickness of the elastic lamina. We haven't done quantitative morphometry on that, nor the biochemistry; until we have it's hard to say. It is, however, possible that, even though these cells are secreting a lot more tropoelastin, it's not incorporated, because you're saturating a fibre assembly process which cannot cope with any more, and the rest is getting degraded.

Parks: Have you considered looking for tropoelastin peptides circulating in blood or urine?

Boyd: Yes, we are starting such a study.

Keeley: We know from our stretch experiments in aortic tissue that the rate of synthesis of elastin can be almost doubled, without any increases in steady-state levels of tropoelastin in the tissue. In other words, the assembly mechanisms are capable of handling twice the rates of synthesis without tropoelastin backing up and accumulating in the tissue, even in day-old chicks which are already making elastin at a very rapid rate.

Pierce: Is there a theoretical P32:P37 ratio that you could deduce for elastin isolated from your Tropoprom-4 transgenes?

Boyd: Limitations in the assay prevent us from deducing an actual number. We predicted, however, a ratio less than the P32:P37 ratio recovered from mouse elastin. A you will remember from the results I presented in Table 2, that's exactly what we found.

ıge, not pre-message.
in the pre-mRNA or the processed
ıal regulation of tropoelastin are not
hypothesis is that this mechanism is
ɔe you're seeing continued expression
ıe 3′ UTR, I think we might have to
ɾts of the gene, such as intron 1, which
: and is not present in your minigenes.
ɔn; that some intron sequence absent
a post-transcriptional control in pre-
onsistent with the results you've got.
s a process that requires numerous
ise, co-ordinate regulation of these
nodel, you're supplying more of the
Do you think that more elastin will
that affect our thinking about the

is going to be important to answer
us thickening of the elastic fibres (as
of the ascending aorta from the
a huge increase in the total amount
assembly in those animals, but we
ɛss of the elastic lamina. We haven't
ıor the biochemistry; until we have
that, even though these cells are
not incorporated, because you're
ı cannot cope with any more, and

tropoelastin peptides circulating in

ɾiments in aortic tissue that the rate
ed, without any increases in steady-
e. In other words, the assembly
ice the rates of synthesis without
in the tissue, even in day-old chicks
rapid rate.
ıio that you could deduce for elastin
ɛs?
s from deducing an actual number.
the P32:P37 ratio recovered from
the results I presented in Table 2,

Pierce: That would indicate that there was overwhelming production primarily of the transgene as opposed to the endogenous gene.

Boyd: I would be cautious about making a quantitative judgement on those HPLC assays. One thing we've learnt from Larry Sandberg is that it's hard to say exactly how much tropoelastin from any one of the transgenes will contribute to the P32:P37 ratio. All we're saying is that the ratio is different enough from the endogenous mouse to illustrate clearly that there is rat tropoelastin present in transgenic mouse elastin.

Davidson: Do you have any information to indicate whether it's pre-transcriptional, transcriptional or post-transcriptional control that regulates these mRNA levels?

Boyd: No; that's obviously a priority.

1/14/00
Chen, Shih-Lin
1633

SO JOURNAL OF CLINICAL INVESTIGATION, (15 NOV 1998) Vol. 102, No. 10, pp. 1788-1797.
Publisher: ROCKEFELLER UNIV PRESS, 1114 FIRST AVE, 4TH FL, NEW YORK, NY 10021.
ISSN: 0021-9738.

L11 ANSWER 22 OF 54 SCISEARCH COPYRIGHT 2000 ISI (R)
AU TAKEUCHI T (Reprint); GASPARRO F P; BROWN D B; KONG S K; LOPRESTI N; WHITE T; CHANG P; UITTO J; BERNSTEIN E F
TI 8-METHOXYPSORALEN AND ULTRAVIOLET A RADIATION ACTIVATE THE HUMAN ***ELASTIN*** PROMOTER IN ***TRANSGENIC*** ***MICE*** - IN-VIVO AND IN-VITRO EVIDENCE FOR GENE INDUCTION
SO JOURNAL OF INVESTIGATIVE DERMATOLOGY, (APR 1996) Vol. 106, No. 4, pp. 543.
ISSN: 0022-202X.

L11 ANSWER 26 OF 54 SCISEARCH COPYRIGHT 2000 ISI (R)
AU KATCHMAN S (Reprint); WU M; COVELLO S; UITTO J
TI GLUCOCORTICOSTEROIDS UP-REGULATE HUMAN ***ELASTIN*** PROMOTER ACTIVITY IN THE SKIN OF ***TRANSGENIC*** ***MICE*** IN AN AGE-INDEPENDENT MANNER
SO JOURNAL OF INVESTIGATIVE DERMATOLOGY, (APR 1996) Vol. 106, No. 4, pp. 204.
ISSN: 0022-202X.

L11 ANSWER 29 OF 54 MEDLINE DUPLICATE 11
AU Katchman S D; Del Monaco M; Wu M; Brown D; Hsu-Wong S; Uitto J
TI A ***transgenic*** ***mouse*** model provides a novel biological assay of topical glucocorticosteroid potency.
SO ARCHIVES OF DERMATOLOGY, (1995 Nov) 131 (11) 1274-8.
Journal code: 6WU. ISSN: 0003-987X.

L11 ANSWER 31 OF 54 BIOSIS COPYRIGHT 2000 BIOSIS DUPLICATE 13
AU Del Monaco, Magaly; Wu, May; Katchman, Stacy; Tan, Elaine M. L.; Uitto, Jouni
TI Basic fibroblast growth factor regulates human ***elastin*** promoter activity in ***transgenic*** ***mice*** .
SO Journal of Investigative Dermatology, (1995) Vol. 104, No. 4, pp. 580.
Meeting Info.: Annual Meeting of the Society for Investigative Dermatology Chicago, Illinois, USA May 24-28, 1995
ISSN: 0022-202X.

L11 ANSWER 44 OF 54 MEDLINE DUPLICATE 23
AU Reitamo S; Remitz A; Tamai K; Ledo I; Uitto J
TI Interleukin 10 up-regulates ***elastin*** gene expression in vivo and in vitro at the transcriptional level.
SO BIOCHEMICAL JOURNAL, (1994 Sep 1) 302 (Pt 2) 331-3.
Journal code: 9YO. ISSN: 0264-6021.

L11 ANSWER 47 OF 54 BIOSIS COPYRIGHT 2000 BIOSIS
AU Ledo, Isabel; Mauviel, Alain; Wu, May; Hsu-Wong, Sylvia; Uitto, Jouni
TI Use of a ***transgenic*** ***mouse*** model to study the regulation of the human ***elastin*** promoter activity in vivo.
SO Journal of Investigative Dermatology, (1993) Vol. 100, No. 4, pp. 510.
Meeting Info.: Annual Meeting of the Society for Investigative Dermatology Washington, D.C., USA April 28-May 1, 1993
ISSN: 0022-202X.

MIC
or
RL1.J8

128

IDENTIFICATION OF MYCOBACTERIAL DNA IN CUTANEOUS TUBERCULOSIS AND SARCOIDOSIS BY THE POLYMERASE-CHAIN REACTION (PCR). K. Degitz, M. Steidl, U. Neubert, M. Volkenandt, G. Plewig. Department of Dermatology, Ludwig-Maximilians University, Munich, Germany.

Tuberculosis is a resurgent medical problem, and cutaneous tuberculosis remains an important differential diagnosis of granulomatous skin diseases. The detection of mycobacteria in skin lesions has remained difficult. Acid-fast bacteria are rarely detected histologically, and the culture of mycobacteria takes many weeks. We have established a system for the detection of mycobacterial DNA from routinely prepared formalin-fixed, paraffin-embedded skin specimens using PCR. Results can be obtained within a few days. DNA is isolated by dissolving paraffin in xylene, pronase digestion of deparaffinized tissue, protein extraction, and ethanol precipitation. PCR is performed using previously described primers (Hance AJ et al., Mol. Microbiol.3:843) amplifying a 383 bp fragment of the mycobacterial groEL gene. The amplification primers anneal to sequences conserved between mycobacteria. Based on a sequence heterogeneity within the amplified segment, oligonucleotides differentially hybridizing to Mycobacterium-tuberculosis (Mt) complex DNA (TB-4) or M.-avium DNA (TB-5) are used for species-specific hybridization with PCR products in Southern blots. PCR-negative samples are checked for PCR-inhibiting factors by PCR amplification of an external control gene. PCR using DNA of a Mt isolate as template generated a product hybridizing with TB-4. PCR using DNA of normal skin was negative (n=8). In 8 of 15 specimens of lupus vulgaris, Mt complex DNA was detected. 5 of 7 specimens from lesions of Erythema induratum of Bazin and 4 of 6 of papulonecrotic tuberculid also contained Mt complex DNA. 6 of 14 specimens of lesions of cutaneous sarcoidosis were positive for Mt complex DNA, but none for DNA of M. avium. Conclusions: 1) PCR based detection of Mt complex DNA might significantly accelerate the diagnosis of tuberculosis. 2) Some so called tuberculids may rather be viewed as forms of post-primary tuberculosis. 3) The detection of mycobacterial DNA in skin lesions reopens the discussion about a mycobacterial etiology of cutaneous sarcoidosis.

129

LACK OF DNA RELEASE WITH COPPER VAPOR LASER TREATMENT OF HUMAN SKIN. Albert J. Nemeth, Craig Leonardi, Christopher Calloway, Dept. of Dermatology and Cutaneous Surgery, Univ. of Miami, FL. We have recently demonstrated the efficacy of green light (511nm) from a Copper Vapor Laser (CVL) in the treatment of anogenital and body warts. Unlike CO2 Laser therapy, no smoke plume is generated with CVL, suggesting no infectious threat. Copper Vapor and CO2 lasers, in seperate treatment rooms, were used to treat fresh human skin obtained from Mohs surgery. All laser parameters duplicated actual treatments. The air and smoke (positive control) was collected from the treatment sites, and from the rooms 3 minutes after the vacuum was turned off (negative control) All air and smoke samples were analyzed for the presence of human Beta-Globin Gene, a marker to detect any DNA release, using the Polymerase Chain Reaction (PCR). The human Beta-Globin Gene is ubiquitous and present in every single human cell in a quantity that is greater than or equal to any infectious agent. Thirty-one (84%) of 37 cases treated with CO2 laser were positive for DNA release. There was no correlation of DNA detection with the wattage or duration of CO2 laser treatment ie.e. no safe range was found. None (0%) of 55 CVL cases were positive. None of the 20 air samples collected 3 minutes after completion of 20 cases in the seperate treatment rooms were positive. The lack of any DNA detection with CVL strongly suggests CVL poses no infectious threat. Our study strongly suggests CVL is a much safer alternative than CO2 Laser therapy.

130

SKIN DRYNESS - WHAT IS IT?. +A. Rawlings, #J. Hope, #J. Rogers, #A. Mayo, #A. Watkinson and +I. Scott, +Unilever Research, Edgewater, NJ, USA, #Unilever Research, Colworth House, Sharnbrook, Bedford, UK

To understand skin dryness, we have compared sequential human tape strippings of healthy and dry skin. We have used electron microscopy to examine the morphology of desmosomes and lipid organization, high performance thin layer chromatography to examine lipid composition, and electrophoretic methods to examine desmosomal proteins.

In healthy skin, desmosomes were shown to be degraded towards the surface of the stratum corneum when viewed by electron microscopy. Their degradation was confirmed by measuring the reduction in desmoglein I (dgI) levels. In skin dryness, desmosomes remained intact in the skins surface layers and dgI levels remained elevated. (Normal skin; dgI/keratin = 0.218 ± 0.27; dry skin, dgI/keratin = 3.94 ± 2.93; $P < 0.05$) . In addition, the normal bilayer structure of lipids was totally perturbed in skin dryness. This change in lipid molecular architecture was associated with increases in stratum corneum fatty acid levels and decreases in ceramide levels. (Percentage of fatty acids, normal skin = 46.2 ± 9.8; dry skin = 56.0 ± 10.8; percentage of ceramides normal skin = 48.3 ± 8.6; dry skin = 38.3 ± 11.2).

From these results we conclude that desquamation proceeds by desmosomal digestion. This process is disrupted in skin dryness and is associated with changes in stratum corneum lipid organization and composition.

131

TARGETED EXPRESSION OF TRANSFORMING GROWTH FACTOR-β_1 (TGF-β_1) TO THE EPIDERMIS LEADS TO LIVEBORN, BUT NON-VIABLE TRANSGENIC MICE. K. Sellheyer, J. Rothnagel, D. Bundman, M. Longley, A. Geiser, A. Roberts, and D. Roop, National Institutes of Health, Bethesda, MD; Baylor College of Medicine, Houston, TX

TGF-β is a peptide growth factor involved in a variety of biological processes, such as embryogenesis, carcinogenesis, immunomodulation, wound healing and tissue repair. TGF-β often has seemingly opposite effects on different cell types within a given organ. In the skin, for example, in vitro studies have shown that TGF-β induces proliferation of dermal fibroblasts, but growth arrest of epidermal keratinocytes. To assess the effect of TGF-β on the skin in vivo, we have targeted its expression to the epidermis of transgenic mice. To ensure that active TGF-β was expressed, we utilized a porcine TGF-β_1 cDNA with two site-specific mutations of cysteine to serine at positions 223 and 225. These mutations allow constitutive activation of TGF-β_1 from its latent stage in which mature TGF-β_1 is bound to the latency associated peptide (N-terminal remnant of the TGF-β_1 precursor) by disulfide bonds. Mice expressing the mutant TGF-β_1 transgene exhibited a marked phenotype at birth. The skin was very shiny and tautly stretched. The dermatoglyphic pattern seen on normal littermates was not apparent on the TGF-β_1 mice. These animals were rigid and appeared to be restricted in their ability to move and breathe. Death occurred within approximately 24 hours. Histologically, the most prominent feature of the epidermis was a compact orthohyperkeratosis. There was a reduction in the number of hair follicles, however, the dermis appeared to be unaltered. Although these results demonstrate that constitutive expression of TGF-β_1 adversely affects epidermal development, restricted expression of active forms of TGF-β_1 could be beneficial in the treatment of hyperproliferative skin disorders.

132

ANALYSIS OF TGF-ß FUNCTION IN THE EPIDERMIS USING TGF-ß1 KNOCKOUT MICE. Adam B. Glick, Ashok B. Kulkarni*, Tamar Tennenbaum, Stefan Karlsson*, and Stuart H. Yuspa, Laboratory of Cellular Carcinogenesis and Tumor Promotion, National Cancer Institute and *Molecular Medical Genetics Section, National Institute of Neurological Disorders and Stroke, Bethesda, Maryland.

The transforming growth factor-ß's inhibit keratinocyte proliferation, and regulate extracellular matrix gene expression in vitro. In mouse epidermis, TGF-ß1 is expressed in the basal layer and TGF-ß2 in the suprabasal strata. To study the function of endogenous TGF-ß, a TGF-ß1 replacement vector was used to disrupt the TGF-ß1 allele in embryonic stem cells, and mice homozygous for a TGF-ß1 null allele were bred from the resulting chimaeras. Analysis of skin from 3-4 week old TGF-ß1 null mice by indirect immunofluorescence showed that TGF-ß1 was absent, but that TGF-ß2 was expressed at wild type levels. Despite the continued expression of TGF-ß2, the epidermis of these mice was hyperproliferative, with a 3-4 fold increase in labelling index, but was not hyperplastic. Keratins 1 and 10 were expressed normally, and keratins 13 and 6 were not expressed. Expression of the integrin α6 which is localized to the basement membrane face of the basal layer was markedly increased in the TGF-ß1 null epidermis. These results show that TGF-ß1 is an endogenous negative regulator of keratinocyte proliferation and integrin expression, and that TGF-ß1 and TGF-ß2 may have distinct compartmentalized functions.

133

USE OF A TRANSGENIC MOUSE MODEL TO STUDY THE REGULATION OF THE HUMAN ELASTIN PROMOTER ACTIVITY *IN VIVO*. Isabel Ledo, Alain Mauviel, May Wu, Sylvia Hsu-Wong, and Jouni Uitto, Department of Dermatology, Jefferson Medical College, Philadelphia, Pennsylvania.

We have developed a homologous line of transgenic mice which express 5.2 kb of human elastin promoter linked to the CAT reporter gene in a tissue specific manner, as compared to the endogenous elastin gene. This transgenic model allows precise *in vivo* studies on the regulation of the human elastin promoter. To investigate the role of interleukin (IL)-1 on elastin gene expression in the skin, recombinant human (rh) IL-1β (100 U) was injected subcutaneously in the back of 5d-old animals, and 24hrs later, the activity of the transgene was measured in the skin. The results demonstrated up-regulation (~4-fold) of the CAT activity by IL-1β. Furthermore, different fibroblast cell cultures were established from the transgenic animals by explanting specimens from the skin and the lungs. Incubation of these cells for 40 hrs with various concentrations of rhIL-β resulted in a dose-dependent up-regulation of CAT activity, reflecting activation of the transgenic human elastin promoter by IL-1. Also similar observations were made in transient transfection experiments of human dermal fibroblast cultures with the chimeric gene construct used to develop the transgenic mice. Taken together, these data indicate that IL-1 up-regulates elastin gene expression *in vivo* and *in vitro*, and this activation occurs at the transcriptional level.

SO JOURNAL OF CLINICAL INVESTIGATION, (15 NOV 1998) Vol. 102, No. 10, pp. 1788-1797.
Publisher: ROCKEFELLER UNIV PRESS, 1114 FIRST AVE, 4TH FL, NEW YORK, NY 10021.
ISSN: 0021-9738.

1/14/00
Chen, Shih-Lin
1633

L11 ANSWER 22 OF 54 SCISEARCH COPYRIGHT 2000 ISI (R)
AU TAKEUCHI T (Reprint); GASPARRO F P; BROWN D B; KONG S K; LOPRESTI N; WHITE T; CHANG P; UITTO J; BERNSTEIN E F
TI 8-METHOXYPSORALEN AND ULTRAVIOLET A RADIATION ACTIVATE THE HUMAN ***ELASTIN*** PROMOTER IN ***TRANSGENIC*** ***MICE*** - IN-VIVO AND IN-VITRO EVIDENCE FOR GENE INDUCTION
SO JOURNAL OF INVESTIGATIVE DERMATOLOGY, (APR 1996) Vol. 106, No. 4, pp. 543.
ISSN: 0022-202X.

L11 ANSWER 26 OF 54 SCISEARCH COPYRIGHT 2000 ISI (R)
AU KATCHMAN S (Reprint); WU M; COVELLO S; UITTO J
TI GLUCOCORTICOSTEROIDS UP-REGULATE HUMAN ***ELASTIN*** PROMOTER ACTIVITY IN THE SKIN OF ***TRANSGENIC*** ***MICE*** IN AN AGE-INDEPENDENT MANNER
SO JOURNAL OF INVESTIGATIVE DERMATOLOGY, (APR 1996) Vol. 106, No. 4, pp. 204.
ISSN: 0022-202X.

L11 ANSWER 29 OF 54 MEDLINE DUPLICATE 11
AU Katchman S D; Del Monaco M; Wu M; Brown D; Hsu-Wong S; Uitto J
TI A ***transgenic*** ***mouse*** model provides a novel biological assay of topical glucocorticosteroid potency.
SO ARCHIVES OF DERMATOLOGY, (1995 Nov) 131 (11) 1274-8.
Journal code: 6WU. ISSN: 0003-987X.

L11 ANSWER 31 OF 54 BIOSIS COPYRIGHT 2000 BIOSIS DUPLICATE 13
AU Del Monaco, Magaly; Wu, May; Katchman, Stacy; Tan, Elaine M. L.; Uitto, Jouni
TI Basic fibroblast growth factor regulates human ***elastin*** promoter activity in ***transgenic*** ***mice*** .
SO Journal of Investigative Dermatology, (1995) Vol. 104, No. 4, pp. 580.
Meeting Info.: Annual Meeting of the Society for Investigative Dermatology Chicago, Illinois, USA May 24-28, 1995
ISSN: 0022-202X.

MIC
or
RL1.J8

L11 ANSWER 44 OF 54 MEDLINE DUPLICATE 23
AU Reitamo S; Remitz A; Tamai K; Ledo I; Uitto J
TI Interleukin 10 up-regulates ***elastin*** gene expression in vivo and in vitro at the transcriptional level.
SO BIOCHEMICAL JOURNAL, (1994 Sep 1) 302 (Pt 2) 331-3.
Journal code: 9YO. ISSN: 0264-6021.

L11 ANSWER 47 OF 54 BIOSIS COPYRIGHT 2000 BIOSIS
AU Ledo, Isabel; Mauviel, Alain; Wu, May; Hsu-Wong, Sylvia; Uitto, Jouni
TI Use of a ***transgenic*** ***mouse*** model to study the regulation of the human ***elastin*** promoter activity in vivo.
SO Journal of Investigative Dermatology, (1993) Vol. 100, No. 4, pp. 510.
Meeting Info.: Annual Meeting of the Society for Investigative Dermatology Washington, D.C., USA April 28-May 1, 1993
ISSN: 0022-202X.

151

AP-1, BUT NOT NF-κB, MEDIATES LPS UPREGULATION OF COLLAGENASE. Richard A. Pierce, Stephanie Sandefur, Glenn A. Doyle, and Howard G. Welgus, Division of Dermatology, Department of Medicine, Washington University Medical School at Jewish Hospital, St. Louis, MO 63110

Interstitial collagenase, a metalloproteinase produced by resident and inflammatory cells during wound healing, cleaves fibrillar collagen. This step is rate-limiting in remodeling of tissues rich in fibrillar collagen such as skin. Bacterial lipopolysaccharide (LPS) is a potent, cell-type-specific inducer of collagenase transcription in macrophages. In many inflammatory responses, both AP-1 and NF-κB mediate transcriptional regulation of early-response genes. Little is known, however, of the transcriptional elements controlling collagenase transcription in macrophages. To delineate LPS-responsive cis-acting elements of the collagenase promoter, we transiently transfected phorbol-ester (PMA)-differentiated U937 cells with a series of collagenase promoter-CAT constructs. Cells were treated with LPS for 24 h, then cell lysates were assayed for CAT activity. Marked increases in CAT activity were noted in LPS-treated cells transfected with deletion constructs containing from 2278 to 95 bp of the 5'-flanking region of the collagenase promoter, indicating LPS-responsive elements reside near the transcription start site. Further analysis showed a construct spanning -72 to +36 was sufficient for LPS-mediated transcriptional induction. Mutagenesis of the AP-1 site at -72 abolished basal promoter activity and LPS-inducibility. In contrast, mutagenesis of an NF-κB-like site at -19 had no effect on basal activity or LPS-inducibility. Gel-shift analysis showed increased specific binding to the collagenase AP-1 site, but not the NF-κB-like site, in nuclear extracts from LPS-treated cells. Supershift analysis with Fos and Jun-specific antibodies showed the AP-1 complexes contained proteins of both the *jun* and *fos* gene families. These data indicate that AP-1, but not NF-kB, mediates LPS induction of collagenase transcription in macrophage-like cells.

152

GELATINASE B GENE EXPRESSION BY KERATINOCYTES IS REGULATED BY TRANSCRIPTION FACTOR AP-2. M. Elizabeth Fini, John D. Bartlett, Ruchi Gupta, William B. Rinehart, Peter M. Sadow, Judith A. West-Mays, *Trevor Williams and †James D. Zieske. Cutaneous Biology Research Center, Mass. General Hospital and Dept. Dermatology, Harvard Medical School, Charlestown, MA; Dept. Biology, Yale University, New Haven, CT; †Schepens Eye Research Institute, Boston, MA

We have been investigating molecular mechanisms regulating tissue repair. The matrix metalloproteinase, gelatinase B, is not expressed in uninjured stratified epithelia, however, expression occurs in epithelial basal cells at the leading edge of healing corneal and skin wounds. The transcriptional promoter of the gelatinase B gene has, therefore, served as our focus for study. Examination of the sequences upstream of the transcription start site of the rabbit gene revealed several potential binding sites for the transcription factor, AP-2, which has been implicated in epithelial-specific gene expression. Transfection of an AP-2 expression construct into a rabbit epithelial cell line (SIRC), which normally expresses AP-2 at only low levels, stimulated expression of the endogenous gelatinase B gene. The AP-2 construct also stimulated expression of a co-transfected reporter construct driven by 522bp of the gelatinase B promoter. When the reporter construct was introduced into primary keratinocyte cultures, along with an expression construct for an AP-2 interfering form, reporter expression was inhibited. Immunolocalization studies indicated that AP-2 is expressed specifically in basal cells of the corneal epithelium, in situ, and expression appears to be upregulated in these cells at the leading edge of the epithelium migrating to heal a corneal wound. Interestingly, resurfacing epithelial cells that are not in contact with the underlying matrix (due to wound irregularities) express very little AP-2. These results suggest that AP-2 mediates reciprocal interations between keratinocytes and their matrix. Such a mechanism may be responsible for controlling gene expression according to a precise spatial pattern in repairing stratified epithelia.

153

BASIC FIBROBLAST GROWTH FACTOR REGULATES HUMAN ELASTIN PROMOTER ACTIVITY IN TRANSGENIC MICE. Magaly Del Monaco, May Wu, Stacy Katchman, Elaine M.L. Tan, and Jouni Uitto, Thomas Jefferson University, Philadelphia, PA.

We have recently developed a homozygous line of transgenic mice which express human elastin/ CAT reporter gene construct in a tissue-specific and developmentally regulated manner. These mice serve as a model for transcriptional regulation of the human elastin gene at the promoter level. Various cytokines have been previously shown to affect the regulation of the human elastin gene. Basic fibroblast growth factor (bFGF) is a multi-functional peptide present in virtually all cell types, demonstrating angiogenic, neurotropic, and mesoderm-inducing properties. In this study we examined the effects of bFGF in these transgenic mice, and cells derived from them, both *in vivo* and *in vitro*. Five-day old mice were injected subcutaneously with different concentrations of bFGF. Upregulation of the human elastin promoter was seen in a time- and dose-dependent manner. Maximal effect was seen at 72 hrs post-treatment, with a 5.4-fold increase in CAT activity being noted over the control with 200 ng of bFGF. Similarly, upregulation was seen in aortic smooth muscle and lung fibroblast cell cultures incubated with bFGF . In aortic smooth muscle cell culture, there was an 18.4-fold increase over the control in CAT activity with 50 ng/ml of bFGF. Lung fibroblasts, showed a less dramatic increase in CAT activity, while skin fibroblasts in culture treated with bFGF did not show an increase in CAT activity. These results suggest that bFGF has a regulatory effect on human elastin gene expression in a tissue specific manner.

154

DIFFERENTIAL EXPRESSION OF MATRIX METALLOPROTEINASES BY MICROVASCULAR ENDOTHELIAL CELLS DURING DIFFERENT STAGES OF ANGIOGENESIS. L.A. Cornelius*, L.C. Nehring*, J. Roby*, W. Frazier[1], W.C. Parks*, H.G. Welgus*, *Dermatology Division at Jewish Hospital and [1]Department of Biochemistry and Molecular Biophysics, Washington University School of Medicine, St. Louis, MO.

Wound healing and angiogenesis are interrelated processes which involve the interplay of cellular and matrix components. *In vivo*, microvascular endothelial cells (MECs) reside upon an intact basement membrane. Matrix metalloproteinases (MMPs) are inducible matrix-degrading enzymes which enable the cells which produce them to traverse through basement membrane and interstitial matrix. Early events of angiogenesis involve MEC migration within a provisional matrix composed of interstitial matrix proteins, as well as inflammatory mediators and growth factors prior to their actual formation of intact vessels. We undertook studies to test our hypothesis that during the early phases of angiogenesis, or cell migration, MECs express certain MMPs that are distinct from those involved in the later phases, or tube formation. In an attempt to reproduce the early *in vivo* situation, MECs were plated to confluence and then allowed to migrate onto type I collagen. Employing immunohistochemistry, we found an increase in cell-associated collagenase expression by the migrating cells, which was augmented by two physiologically relevant angiogenic factors, basic fibroblast growth factor (bFGF) and vascular endothelial cell growth factor (VEGF). Additionally, in an *in vitro* assay representative of later stages of angiogenesis, bFGF-treated MECs induced to form tubes on type I collagen preferentially expressed 92 kDa gelatinase. Using ELISAs and immuno-precipitation (IP) assays, we have found not only a separate, but an additive upregulation of secreted MEC collagenase by bFGF and VEGF. Conversely, thrombospondin, an inhibitor of angiogenesis, inhibited MEC collagenase expression. In sum, we have demonstrated the selective expression and regulation of MEC MMP expression not only by agents physiologically relevant to angiogenesis but, most importantly, by the phase of endothelial cell differentiation itself.

155

INTERSTITIAL COLLAGENASE IN BLOOD VESSEL DEVELOPMENT IN FETAL SKIN AND CUTANEOUS TUMORS. T.V. Karelina, G.I. Goldberg, A.Z. Eisen Division of Dermatology, Washington University School of Medicine, St. Louis, Missouri.

In vitro angiogenesis models suggest that new blood vessel formation requires the ability of endothelial cells to induce proteolytic degradation of a surrounding extracellular matrix (ECM), and that secretion of ECM metalloproteases by these cells may be a major mechanism involved in ECM proteolysis. The results of these in vitro studies cannot be directly extrapolated to the process of in vivo angiogenesis because the type of ECM employed and the repertoire of the enzymes secreted by the cells in vitro differs dramatically from the in vivo conditions. To investigate the in vivo role of ECM metalloproteases in blood vessel development, we looked for evidence of elevated expression of these proteases in endothelial cells involved in fetal angiogenesis and neovascularization of certain invasive skin tumors. In situ localization using specific antibodies against ECM metalloproteases showed that in fetal tissue, interstitial collagenase (CI) was the major enzyme involved in de novo blood vessel formation from undifferentiated mesoderm. Early vessel formation in fetal skin was characterized by clusters of CI-positive endothelial cells that subsequently differentiate into vascular tubes surrounded by type IV collagen. Vessel elongation and capillary sprouting were observed as major morphological manifestations of angiogenesis in fetal tissues and was characterized by the presence of CI-positive endothelial cells. In contrast to less aggressive skin tumors in which only a few CI-positive blood vessels were seen and there was no evidence of neovascularization, in aggressive cutaneous tumors, i.e. recurrent basal cell carcinoma and squamous cell carcinomas, there was active neovascularization associated with a marked increase in the number of CI-positive vessels. Numerous short arrays of CI-positive endothelial cells originating from the ends of elongated vessels were seen extending into the tumor nests. Immunofluorescent staining failed to detect stromelysin, matrilysin and 72- and 92-kDa type IV collagenase (gelatinase) in fetal or tumor blood vessels.

These findings are consistent with the hypothesis that proteolytic degradation of the ECM by migrating endothelial cells is required for the formation of new blood vessels. Our results demonstrate that interstitial collagenase rather than other metalloproteinases play an important role in this process.

156

CONTACT WITH NATIVE TYPE I COLLAGEN INDUCES COLLAGENASE PRODUCTION IN MIGRATING KERATINOCYTES. Barry D. Sudbeck, Brian K. Pilcher, Howard G. Welgus, and William C. Parks. Dermatology Division, Jewish Hospital, Washington University Medical School, St. Louis, Missouri

Metalloproteinases are a structurally related family of matrix-degrading proteases involved in tissue remodeling. Among these, interstitial collagenase is unique in that it cleaves fibrillar type I collagen making this matrix protein susceptible to further digestion by other proteases. We have shown in a variety chronic and acute human wounds that collagenase is predominantly and invariantly expressed by basal keratinocytes which are not in contact with the basement membrane but rather are migrating across the dermal matrix. These observations suggest that induction of collagenase expression by migrating keratinocytes is influenced by altered cell:matrix interactions. Reflecting these *in vivo* observations, collagenase production was induced in primary human keratinocytes grown on various preparations of native type I collagen, but only background levels of enzyme were detected in cells grown on 40°C- or 80°C-denatured collagen (gelatin) or on enzymatically-fragmented collagen. Basement membrane proteins (Matrigel) or other matrix proteins found in the wound environment, such as laminin, fibrin, or fibronectin, either did not induce collagenase expression or did not support keratinocyte attachment. As demonstrated by transient transfection with a collagenase promoter-CAT expression vector and by Northern hybridization, collagen-mediated induction of collagenase in keratinocytes was transcriptionally controlled. As suggested by an *in vitro* migration assay, collagenase may aid in dissociating keratinocytes from the collagen-rich matrix and promote cell locomotion across the dermis. Indeed, collagenase-deficient HaCat cells did not migrate on collagen, but they did move efficiently on gelatin. Thus, the expression of collagenase by basal keratinocytes is a common and programmed response to skin wounding, and the collagenolytic activity of this metalloproteinase may be needed for proper and efficient re-epithelialization.

SO JOURNAL OF CLINICAL INVESTIGATION, (15 NOV 1998) Vol. 102, No. 10, pp. 1788-1797.
Publisher: ROCKEFELLER UNIV PRESS, 1114 FIRST AVE, 4TH FL, NEW YORK, NY 10021.
ISSN: 0021-9738.

1/14/00
Chen, Shin-Lin
1633

L11 ANSWER 22 OF 54 SCISEARCH COPYRIGHT 2000 ISI (R)
AU TAKEUCHI T (Reprint); GASPARRO F P; BROWN D B; KONG S K; LOPRESTI N; WHITE T; CHANG P; UITTO J; BERNSTEIN E F
TI 8-METHOXYPSORALEN AND ULTRAVIOLET A RADIATION ACTIVATE THE HUMAN ***ELASTIN*** PROMOTER IN ***TRANSGENIC*** ***MICE*** - IN-VIVO AND IN-VITRO EVIDENCE FOR GENE INDUCTION
SO JOURNAL OF INVESTIGATIVE DERMATOLOGY, (APR 1996) Vol. 106, No. 4, pp. 543.
ISSN: 0022-202X.

L11 ANSWER 26 OF 54 SCISEARCH COPYRIGHT 2000 ISI (R)
AU KATCHMAN S (Reprint); WU M; COVELLO S; UITTO J
TI GLUCOCORTICOSTEROIDS UP-REGULATE HUMAN ***ELASTIN*** PROMOTER ACTIVITY IN THE SKIN OF ***TRANSGENIC*** ***MICE*** IN AN AGE-INDEPENDENT MANNER
SO JOURNAL OF INVESTIGATIVE DERMATOLOGY, (APR 1996) Vol. 106, No. 4, pp. 204.
ISSN: 0022-202X.

MIC
OV
RL1.J8

L11 ANSWER 29 OF 54 MEDLINE DUPLICATE 11
AU Katchman S D; Del Monaco M; Wu M; Brown D; Hsu-Wong S; Uitto J
TI A ***transgenic*** ***mouse*** model provides a novel biological assay of topical glucocorticosteroid potency.
SO ARCHIVES OF DERMATOLOGY, (1995 Nov) 131 (11) 1274-8.
Journal code: 6WU. ISSN: 0003-987X.

L11 ANSWER 31 OF 54 BIOSIS COPYRIGHT 2000 BIOSIS DUPLICATE 13
AU Del Monaco, Magaly; Wu, May; Katchman, Stacy; Tan, Elaine M. L.; Uitto, Jouni
TI Basic fibroblast growth factor regulates human ***elastin*** promoter activity in ***transgenic*** ***mice*** .
SO Journal of Investigative Dermatology, (1995) Vol. 104, No. 4, pp. 580.
Meeting Info.: Annual Meeting of the Society for Investigative Dermatology Chicago, Illinois, USA May 24-28, 1995
ISSN: 0022-202X.

L11 ANSWER 44 OF 54 MEDLINE DUPLICATE 23
AU Reitamo S; Remitz A; Tamai K; Ledo I; Uitto J
TI Interleukin 10 up-regulates ***elastin*** gene expression in vivo and in vitro at the transcriptional level.
SO BIOCHEMICAL JOURNAL, (1994 Sep 1) 302 (Pt 2) 331-3.
Journal code: 9YO. ISSN: 0264-6021.

L11 ANSWER 47 OF 54 BIOSIS COPYRIGHT 2000 BIOSIS
AU Ledo, Isabel; Mauviel, Alain; Wu, May; Hsu-Wong, Sylvia; Uitto, Jouni
TI Use of a ***transgenic*** ***mouse*** model to study the regulation of the human ***elastin*** promoter activity in vivo.
SO Journal of Investigative Dermatology, (1993) Vol. 100, No. 4, pp. 510.
Meeting Info.: Annual Meeting of the Society for Investigative Dermatology Washington, D.C., USA April 28-May 1, 1993
ISSN: 0022-202X.

199

AP1 ACTIVITY REGULATES THE SPECIFICITY OF EXPRESSION FROM THE PROXIMAL PROMOTER OF THE HUMAN PROFILAGGRIN GENE IN CULTURED KERATINOCYTES. S.-I. Inng, P.M. Steinert, and N.G. Markova, Laboratory of Skin Biology, NIAMS, NIH, Bethesda, Maryland 20892-2755

The human profilaggrin gene is expressed in terminally differentiating epidermal cells *in vivo* and in cultured normal human epidermal keratinocytes *in vitro*. We have established that an AP1 recognition motif in the vicinity of the transcriptional initiation site plays a critical role in the keratinocyte specificity of the expression. Deletion of this AP1 motif in the context of the 5' upstream sequences up to position -1532 invariably reduces the expression by more than two-fold and obliterates the keratinocyte specificty of the transcription. Bandshift analysis revealed that the AP1 motif interacts with c-jun, jun B and jun D, as well as with c-fos transcription factors in keratinocyte nuclear extracts. No binding to fra 1, fra 2 and fos B proteins was observed. To elucidate the role of c-fos and the individual jun proteins the profilaggrin transcription, normal human epidermal keratinocytes were co-transfected with wild type and AP1 deletion profilaggrin constructs and with expression vectors carrying cDNA sequences for c-fos, c-jun, a dominant negative mutant of c-jun with a truncated transactivating domain, and jun B. The forced expression of c-jun resulted in a marked up-regulation of expression from the profilaggrin constructs containing the intact AP1 site but had no effect on the AP1 deletion constructs. Simultaneous co-transfection of c-fos and c-jun had an even more pronounced effect. In contrast, the transactivating mutant of c-jun reduced the level of expression by 90%. The positive effect of c-jun on the profilaggrin transcription was confirmed by forcing expression of c-jun in undifferentiated F9 embryonal carcinoma cells. In simple epithelial HeLa cells, the activity of the profilaggrin constructs did not depend on the AP1 binding and the transactivating function of c-jun was not required. Co-transfection of jun B strongly downregulated transcription from the profilaggrin constructs in all cell types. The effect did not depend on binding at the AP1 site. Thus it appears that AP1 activity plays a complex multifactorial role in the regulation of transcription of the profilaggrin gene and in a cell type specific manner.

200

NF-κB AND AP-1 MODULATION OF HUMAN α2(I) COLLAGEN (COL1A2) PROMOTER ACTIVITY. Kee-Yang Chung, Jouni Uitto, and Alain Mauviel, Department of Dermatology and Cutaneous Biology, Jefferson Medical College, Philadelphia, Pennsylvania.

Elucidation of the *cis*-elements of the COL1A2 gene and their corresponding regulatory transcription factors is an essential step in understanding the cytokine-mediated molecular pathways of extracellular matrix deposition during development and in fibrotic diseases. Toward this aim, using a series of 5' deletion promoter/CAT reporter gene constructs, we have mapped the TGF-β response element to a short region of the COL1A2 promoter, between residues -265 and -241, that contains both an AP-1 and an overlapping NF-κB binding site. This region is sufficient to confer the antagonistic activity of TNF-α on the TGF-β effect. Site-directed mutagenesis indicated that the AP-1, but not the NF-κB binding site, is essential for TGF-β upregulation of COL1A2 promoter activity. However, co-transfection experiments with expression vectors for various NF-κB sub-units, together with COL1A2 promoter/CAT constructs indicate that, (i) basal transcriptional activity is enhanced by p50 but not by p65; (ii) over-expression of p105 subunit results in markedly enhanced TGF-β responsiveness; and (iii) p65 partially prevents TGF-β response. All these effects are lost when the NF-κB binding site is mutated, attesting to their specificity. Collectively, these results suggest that NF-κB, due to the close proximity of the corresponding *cis*-element with the AP-1 binding site, may alter the accessibility of the latter site to AP-1, and consequently modifies the transcriptional activity of the COL1A2 promoter, and its regulation by TGF-β.

201

ROLE OF FLANKING SEQUENCES FOR THE ICAM-1 NF-κB RESPONSE ELEMENT IN TNF-α-MEDIATED UPREGULTION OF GENE EXPRESSION. Lani L.L. Paxton, Liao-Jie Li, Shubhada Naik, Naotaka Shibagaki, and S.Wright Caughman, Emory University School of Medicine, Atlanta, GA 30322.

Cytokine-induced regulated expression of ICAM-1 is a critical component in inflammation and immune responses. We have identified a region of the human ICAM-1 5' regulatory region which is necessary and sufficient for TNF-α-mediated induction of gene expression. This region contains a modified NF-κB binding site which interacts with the NF-κB protein p65 in cotransfection studies and p65 and p50 in mobility supershift assays. Mutational analysis demonstrates that specific 5' and 3' flanking sequences surrounding this NF-κB binding site are necessary for TNF-α-mediated induction of ICAM-1 transcription in C32 melanoma cells and human dermal microvascular endothelial cells. When the decameric ICAM-1 NF-κB site is converted to a consensus NF-κB site, it is fully capable of competing for complex formation in an EMSA while the ICAM-1 NF-κB site alone can not. When this consensus NF-κB site is linked to the ICAM-1 NF-κB flanking sequences and used as a probe, it forms complexes which migrate differently from the wild-type ICAM-1 probe. The decameric ICAM-1 NF-κB site alone and the ICAM-1 NF-κB site with flanking regions compete less efficiently for complex formation than does the consensus NF-κB site alone. In UV crosslinking/SDS-PAGE analysis, protein(s) other than those corresponding to p50 and p65 also bind to both the wild-type ICAM-1 and consensus NF-κB/ICAM-1 flanking sequence probes. On the basis of these studies and the interaction of NF-κB proteins with non-NF-κB proteins in the regulation of other genes, the involvement of an additional nuclear protein that complexes with either NF-κB proteins or with DNA flanking the ICAM-1 NF-κB binding site is proposed.

202

IDENTIFICATION OF THE TNFα RESPONSIVE ELEMENT OF THE HUMAN K6 KERATIN GENE PROMOTER

Mayumi Komine, Irwin M. Freedberg and Miroslav Blumenberg, Perelman Department of Dermatology, New York University Medical Center, NY, NY

TNFα elicits various responses during cutaneous inflammation. One such effect is the induction of keratin K6 expression in inflamed epidermis. Transcription factors activated by TNFα include NFκB/c-rel, C/EBP/NFIL-6, and AP-1. The K6 promoter has two putative AP-1 sites although a construct that we mutated in both sites was induced to the same extent as the wild type, indicating that the AP-1 sites play no role in TNFα signaling. Deletion analysis implicated the DNA sequence from -227 to -213 bp in the K6 gene promoter in TNFα regulation. This region and the adjacent sites bind transcription factors C/EBP/NFIL-6 and NFκB/c-rel and also E2BP protein, as we have found previously.

To characterize further the specific binding proteins and the responsive region involved in TNFα induced inflammation, we introduced several mutations into each binding site. Our results indicate that the C/EBP/NFIL-6 site is most important for induction of K6 expression in keratinocytes. We also co-transfected the K6 promoter with constructs expressing p65, p50 and c-rel, components of the NFκB transcription factor. The co-transfected p65, but not p50 or c-rel, induced K6 expression, which suggests that this factor actually controls transduction of the TNFα signal. To elucidate the controls of the signal transduction pathway, we used inhibitors of PC-PLC, PLA2, and PKC. The results implicated PC-PLC and PLA2, but not PKC as the proximate signals involved in induction of K6 by TNFα in human epidermal keratinocytes.

203

IDENTIFICATION OF NOVEL GLUCOCORTICOID RESPONSE ELEMENTS (GREs) IN HUMAN ELASTIN PROMOTER: NUCLEOTIDE SEQUENCE SPECIFICITY OF THE RECEPTOR BINDING. Magaly Del Monaco, Seana Covello, Gwen Gilinger, May Wu, Gerald Litwack, and Jouni Uitto, Depts. Dermatology and Cutaneous Biology, and Pharmacology, Jefferson Medical College, Philadelphia, Pennsylvania.

Glucocorticosteroids exert their action on gene expression through activation of cytoplasmic receptors which bind to GREs, *cis*-regulatory sequences in the promoter. The consensus GRE consists of two half-sites (underlined), separated by three nucleotides: AGAACAGAGTGTTCT. We have recently cloned the entire human elastin gene, including ~5.2 kb of the 5'-flanking DNA. Nucleotide sequencing of the promoter region disclosed the presence of three putative GREs, with the downstream half-site sequence, TGTTCC, having homology with the consensus GRE, while the upstream half-sites showed no homology. To examine the functionality of these putative GREs in binding the glucocorticosteroid receptors, we performed gel mobility shift assays with synthetic oligomers containing the putative GREs and a recombinant truncated glucocorticosteroid receptor, expressed in baculovirus system, which is constitutively activated due to absence of the ligand binding domain. All three GREs identified in the elastin promoter bound the receptor in a specific manner. The most upstream GRE in the elastin promoter was also shown to compete for binding of the receptor to the consensus GRE. Nonconservative substitution of single nucleotides (positions 1-6) within the downstream half-site of the elastin GRE indicated that mutations in the positions 1-3 had little effect , while mutations in positions 4-6 rendered the oligomer less effective in competing for the binding. These observations indicate that the downstream half-site of GREs in the human elastin promoter is sufficient for receptor binding and certain nucleotides are critical for the efficient binding.

204

GLUCOCORTICOSTEROIDS UPREGULATE HUMAN ELASTIN PROMOTER ACTIVITY IN THE SKIN OF TRANSGENIC MICE IN AN AGE-INDEPENDENT MANNER. Stacy Katchman, May Wu, Seana Covello, and Jouni Uitto, Departments of Dermatology and Cutaneous Biology, and Biochemistry and Molecular Biology, Jefferson Medical College, Philadelphia, PA.

We have recently developed a homozygous transgenic mouse line that expresses 5.2 kb of human elastin promoter linked to a CAT reporter gene in a tissue-specific and developmentally regulated manner. We have also shown that this promoter activity can be upregulated by either subcutaneous or intraperitonsal injection or by topical application of glucocorticosteroids. This effect is mediated through three glucocorticoid responsive elements (GREs) which have been identified within the elastin promoter. The glucocorticosteroid effect was shown to be dose-dependent, and the peak activation of the promoter after a single subcutaneous injection occurred between 12 and 24 hours. Previous indications in the literature have suggested the glucocorticoid responsiveness of human elastin promoter may be limited to the neonatal and early postnatal period. To test the age-dependent responsiveness of the human elastin promoter, transgenic mice, varying from 3 weeks to 20 months of age, were injected with a single subcutaneous injection of dexamethasone (DEX), 10 μg in 200 μl of saline. The injected area of skin was biopsied at 24 hours, and CAT activity as an indication of the elastin promoter activity was determined. The results indicated that DEX consistently enhanced the elastin promoter activity in mice of all different ages tested. Thus, DEX is capable of enhancing human elastin promoter activity in the skin of transgenic mice in an age-independent manner. Since, we have recently proposed this transgenic mouse model as a biological system to test the potency of various topical glucocorticosteroid preparations, the results of these studies imply that this model is likely to be applicable also to the older skin.

1/14/00

Chen, Shin-Lin

1633

SO JOURNAL OF CLINICAL INVESTIGATION, (15 NOV 1998) Vol. 102, No. 10, pp. 1788-1797.
Publisher: ROCKEFELLER UNIV PRESS, 1114 FIRST AVE, 4TH FL, NEW YORK, NY 10021.
ISSN: 0021-9738.

L11 ANSWER 22 OF 54 SCISEARCH COPYRIGHT 2000 ISI (R)
AU TAKEUCHI T (Reprint); GASPARRO F P; BROWN D B; KONG S K; LOPRESTI N; WHITE T; CHANG P; UITTO J; BERNSTEIN E F
TI 8-METHOXYPSORALEN AND ULTRAVIOLET A RADIATION ACTIVATE THE HUMAN ***ELASTIN*** PROMOTER IN ***TRANSGENIC*** ***MICE*** - IN-VIVO AND IN-VITRO EVIDENCE FOR GENE INDUCTION
SO JOURNAL OF INVESTIGATIVE DERMATOLOGY, (APR 1996) Vol. 106, No. 4, pp. 543.
ISSN: 0022-202X.

MIC
or
RL1.J8

L11 ANSWER 26 OF 54 SCISEARCH COPYRIGHT 2000 ISI (R)
AU KATCHMAN S (Reprint); WU M; COVELLO S; UITTO J
TI GLUCOCORTICOSTEROIDS UP-REGULATE HUMAN ***ELASTIN*** PROMOTER ACTIVITY IN THE SKIN OF ***TRANSGENIC*** ***MICE*** IN AN AGE-INDEPENDENT MANNER
SO JOURNAL OF INVESTIGATIVE DERMATOLOGY, (APR 1996) Vol. 106, No. 4, pp. 204.
ISSN: 0022-202X.

L11 ANSWER 29 OF 54 MEDLINE DUPLICATE 11
AU Katchman S D; Del Monaco M; Wu M; Brown D; Hsu-Wong S; Uitto J
TI A ***transgenic*** ***mouse*** model provides a novel biological assay of topical glucocorticosteroid potency.
SO ARCHIVES OF DERMATOLOGY, (1995 Nov) 131 (11) 1274-8.
Journal code: 6WU. ISSN: 0003-987X.

L11 ANSWER 31 OF 54 BIOSIS COPYRIGHT 2000 BIOSIS DUPLICATE 13
AU Del Monaco, Magaly; Wu, May; Katchman, Stacy; Tan, Elaine M. L.; Uitto, Jouni
TI Basic fibroblast growth factor regulates human ***elastin*** promoter activity in ***transgenic*** ***mice*** .
SO Journal of Investigative Dermatology, (1995) Vol. 104, No. 4, pp. 580.
Meeting Info.: Annual Meeting of the Society for Investigative Dermatology Chicago, Illinois, USA May 24-28, 1995
ISSN: 0022-202X.

L11 ANSWER 44 OF 54 MEDLINE DUPLICATE 23
AU Reitamo S; Remitz A; Tamai K; Ledo I; Uitto J
TI Interleukin 10 up-regulates ***elastin*** gene expression in vivo and in vitro at the transcriptional level.
SO BIOCHEMICAL JOURNAL, (1994 Sep 1) 302 (Pt 2) 331-3.
Journal code: 9YO. ISSN: 0264-6021.

L11 ANSWER 47 OF 54 BIOSIS COPYRIGHT 2000 BIOSIS
AU Ledo, Isabel; Mauviel, Alain; Wu, May; Hsu-Wong, Sylvia; Uitto, Jouni
TI Use of a ***transgenic*** ***mouse*** model to study the regulation of the human ***elastin*** promoter activity in vivo.
SO Journal of Investigative Dermatology, (1993) Vol. 100, No. 4, pp. 510.
Meeting Info.: Annual Meeting of the Society for Investigative Dermatology Washington, D.C., USA April 28-May 1, 1993
ISSN: 0022-202X.

541

PGP 9.5 EXPRESSION BY FIBROBLASTS IN HUMAN CUTANEOUS WOUNDS. DS Chin, ML Usui, NS Gibran*, JC Ansel† and JE Olerud. Departments of Medicine (Dermatology) and Surgery*, University of Washington, Seattle, WA, †Department of Dermatology, Emory University, Atlanta, GA.

Previously, we reported immunohistochemical staining of ubiquitin and the neuronal marker PGP 9.5 in fibroblast-like cells within cutaneous wounds. PGP 9.5, originally thought to be neuron specific, is a carboxy-terminal hydrolase that removes ubiquitin from proteins. Ubiquitin plays a role in the degradation of the PDGFB receptor, which is important in the regulation of tissue repair. The purpose of this study was to determine whether cells that stain with antibodies to PGP 9.5 are phenotypic fibroblasts and whether they co-express the PDGFB receptor. We were also interested in whether these cells express PGP 9.5 mRNA.

Fourteen and 21 day incisional human skin wounds and chronic ulcers from patients with diabetes or spinal cord injuries were evaluated. Immunofluorescent double-labeling was performed using antibodies against PGP 9.5 and a fibroblast marker 5B5 which detects prolyl 4-hydroxylase. Double-labeling using antibodies against PGP 9.5 and the PDGFB receptor was also performed. Slides were evaluated using confocal microscopy. Probes for PGP 9.5 were designed and reverse transcriptase PCR *in situ* hybridization was performed to detect mRNA in the tissue sections.

Many, but not all of the stellate cells within the wound beds co-localized PGP 9.5 and 5B5. Cells within the chronic granulation tissue from nonhealing ulcers showed similar co-localization. We also detected co-localization of PGP 9.5 and the PDGFB receptor in many wound bed fibroblasts. RT-PCR *in situ* hybridization showed that many of the wound bed fibroblasts express PGP 9.5 mRNA consistent with the immunostaining of PGP 9.5 protein.

This study shows that, in human wounds, PGP 9.5 positive cells with the morphology of fibroblasts demonstrate 5B5 staining, PDGFB receptor staining, and also express PGP 9.5 mRNA. These findings, together with our previous observations of ubiquitin in these cells, support a role for PGP 9.5 in the degradation of the PDGFB receptor. We speculate that PGP 9.5 may be important in the down regulation of PDGF-induced fibrogenesis.

542

TGF-β AND TNF-α UPREGULATE THE HUMAN TYPE VII COLLAGEN (COL7A1) PROMOTER ACTIVITY VIA DISTINCT REGULATORY SEQUENCES. Laurence Vindevoghel, Koo-Yong Chung, Sirpa Kivirikko, Jouni Uitto, and Alain Mauviel. Department of Dermatology and Cutaneous Biology, Jefferson Medical College, Philadelphia, Pennsylvania.

Type VII collagen (COL7A1) is the major, if not the exclusive, component of the anchoring fibrils, attachment structures which secure the association of the dermal-epidermal basement membrane to the underlying dermis. We have previously reported that TGF-β and TNF-α increase COL7A1 gene expression in human dermal fibroblasts in culture, as measured by Northern blot hybridization and immunodetection of the corresponding antigenic epitopes (Mauviel *et al.*, J. Biol. Chem., 269:25-28,1994). To gain insight into the molecular mechanisms underlying the upregulation of COL7A1 by these cytokines, transient cell transfections with a series of 5'-deletion promoter/chloramphenicol acetyltransferase (CAT) -reporter gene constructs were performed. The region located between nucleotides -524 and -456 was identified as essential for TGF-β response. In contrast, TNF-α response required elements localized closer to the transcription initiation site, between positions -396 and -140 of the promoter. Sequence analysis revealed the existence of an AP-1-like site (TGAATCA) in the region of the putative TGF-β response element, whereas three Sp-1 binding sites and one NF-κB consensus sequence were identified in the putative TNF-α response element region. The characterization of these cytokine/growth factor response elements provides novel insight into the transcriptional regulation of the human type VII collagen gene.

543

8-METHOXYPSORALEN AND ULTRAVIOLET A RADIATION ACTIVATE THE HUMAN ELASTIN PROMOTER IN TRANSGENIC MICE: *IN VIVO* AND *IN VITRO* EVIDENCE FOR GENE INDUCTION. Tsunemichi Takeuchi,[1] Francis P. Gasparro,[2] Douglas B. Brown,[1] Sung K. Kong,[1] Nicholas Lopresti,[1] Terri White,[1] Patrick Chang,[1] Jouni Uitto,[1,3] and Eric F. Bernstein.[1] [1]Department of Dermatology and Cutaneous Biology and [3]Biochemistry and Molecular Biology, Thomas Jefferson University, Philadelphia, PA; [2]Department of Surgery, Yale University School of Medicine, New Haven, CT.

Treatment of skin diseases with the combination of 8-methoxypsoralen and ultraviolet A radiation (PUVA) results in clinical alterations in treated skin which resemble those observed in chronically photodamaged skin. PUVA-treated patients develop non-melanoma skin cancers, pigmentary alterations, and wrinkling characteristic of sun-induced changes. The major alteration in the dermis of sun-damaged skin is the deposition of abnormal elastic fibers, termed solar elastosis, which results from up-regulation of elastin promoter activity in dermal fibroblasts. In order to study photoaging in an experimental system, we utilized a transgenic mouse line which expresses the human elastin promoter/chloramphenicol acetyl transferase construct in a tissue-specific and developmentally regulated manner. Although ultraviolet B radiation has been demonstrated to increase promoter activity *in vitro*, UVA fails to demonstrate a similar effect. In this study, we demonstrate the ability of PUVA treatment to up-regulate elastin promoter activity both *in vitro* and *in vivo*. These data help to explain the development of photoaging in sun-protected PUVA-treated skin. We attribute the up-regulation of elastin promoter activity in response to PUVA to the formation of DNA photoadducts, which do not occur in response to ultraviolet A radiation alone.

544

CONFORMATION, BUT NOT GLYCOSYLATION, IS REQUIRED FOR THE ANTI-PROTEASE ACTIVITY OF NEWLY IDENTIFIED EXTRACELLULAR MATRIX-ASSOCIATED 33 KDA INHIBITOR. C. N. Rao, Yueying Liu, Prasad Reddy, D. J. Reeder, W. Kisiel, D. T. Woodley. Dept. of Dermatology, Northwestern Univ., Chicago, IL; NIST, MD; Dept. of Pathology, Univ. of. NewMexico, NM.

We demonstrated that skin extracellular matrix-associated serine protease inhibitors (Mr = 33,000, 31,000 and 27,000) are biosynthetic products of a single gene with different degrees of glycosylation. 33 kDa is fully glycosylated whereas 31 kDa and 27 kDa are partially glycosylated. The purpose of this study was to assess the role of glycosylation in the anti-protease activity of the mature 33 kDa inhibitor. Full length inhibitor was expressed in *E. coli*, and its activity compared to that of a biologically active eukaryotic recombinant inhibitor (TFPI-2) by reverse zymography. The eukaryotic product inhibited trypsin. However, dithiothreitol abolished the inhibitor activity demonstrating the requirement for disulfide bonds. The recombinant inhibitor from *E. coli* did not inhibit trypsin suggesting a lack of the required disulfide-bonds. To directly assess the role of glycosylation, two approaches were taken. First, fibroblast cultures were treated with tunicamycin and the inhibitor activity assessed by reverse zymography. Secondly, eukaryotic recombinant inhibitor and the 33 kDa inhibitor from fibroblasts were deglycosylated by treatment with N-glycosidase F and the activity of the deglycosylated inhibitors assessed by reverse zymography. In the presence of tunicamycin, the cells secreted a 26 kDa unglycosylated inhibitor which inhibited trypsin. N-glycosidase F released a 26 kDa unglycosylated inhibitor from both eukaryotic recombinant inhibitor and fibroblast inhibitor. The deglycosylated inhibitor retained full anti-protease activity. Based on these results, we conclude that the enzyme inhibitory activity of the novel matrix-associated inhibitors is conformation-dependent but does not require glycosylation.

545

MUTATIONS IN THE TYPE VII COLLAGEN GENE IN DYSTROPHIC EPIDERMOLYSIS BULLOSA, A SPECTRUM OF CLINICAL SEVERITY. D.W Wu, L. Pan, C. T. Tzou, P. Pereira, J. McGuire, G. S. Herron and E.A. Welsh. Department of Dermatology, Stanford University School of Medicine, Stanford, CA.

Dystrophic forms of epidermolysis bullosa (EB) include both dominant (DDEB) and recessive (RDEB) EB. Clinically, patients have chronic erosions, subepidermal blisters and variable scarring. There is a reduction or lack of anchoring fibrils which when present are often malformed. These AFs are composed of type VII collagen (COL7A1), the primary candidate protein in the pathogenesis of both RDEB and DDEB. We have studied a number of different families with disease ranging from severe (lethal) RDEB to a relative mild DEB in which the inheritance pattern is either recessive or a new dominant mutation. We examined genomic DNA from patients and family members for mutations in COL7A1 by PCR amplification of small genomic segments of COL7A1, heteroduplex (MDE) analysis, and nucleotide sequencing of the observed heteroduplexes. In one family with severe RDEB, examination of the PCR fragment corresponding to exon 114 of COL7A1 in the proband (deceased) revealed a heteroduplex in the MDE analysis. Sequence analysis revealed a C-to-T transition at nucleotide 8440 in the NC-2 domain of COL7A1, which converts an arginine residue to a premature termination codon (PTC). This mutation (R2814X) was confirmed in the maternal genomic DNA. Identification of this mutation was used for first trimester DNA-based prenatal diagnosis in this family. Two different patients of consanguineous parentage with moderate to severe DEB have also been analyzed. MDE analysis is indicative of homozygous polymorphisms or mutations in PCR fragments corresponding to exons 74-76 and exons 105-106 in one patient and exons 90-91 in the second patient. A fourth patient with relatively mild DEB of unknown inheritance has also been analyzed in this manner. These studies should help clarify the potential for correlation of molecular defects of DEB with clinical severity.

546

DOWN-REGULATION OF COLLAGEN TYPE VII DEPOSITION AT DERMO-EPIDERMAL JUNCTION(S) (DEJ) OF MORPHOGENETICALLY ACTIVE AREAS IN HUMAN FETAL SKIN. Tatiana V. Karelina, Gregory A. Bannikov, and Arthur Z. Eisen. Division of Dermatology, Washington Univ. School of Medicine, St. Louis, MO.

The behavior of epithelial cell adhesion, migration, and differentiation is controled by interaction at the dermal-epidermal junction (DEJ). Type VII collagen (CVII) is a major collagenous component of anchoring fibrils that plays an essential role in epidermal attachment to the dermis. Here distribution of CVII protein in morphogenetically active areas of the developing epidermis is described using a novel monoclonal antibody specific for CVII. At 7-9 weeks of gestation CVII-immunolabeling was faint and discontinuous. Both the intensity and thickness of the immunostained DEJ gradually increased with fetal age reaching a maximum in adult skin. Two waves of down-regulation of CVII protein deposition were found at the DEJ in the developing epidermis. The first decrease in CVII-immunoreactivity was seen at the DEJ surrounding invading appendageal buds (13-15 weeks). The second decrease in CVII deposition (20-25 weeks) occured at the DEJ surrounding the distal portion of invading appendageal epithelial cords of both hair follicles and sweat glands. When antibodies against type IV collagen or laminin were used, no differences were seen between the DEJ of the budding and resting fetal epidermis and the various portions of fetal hair follicles. Studies were undertaken to determine whether the expression of matrix metalloproteinases (MMPs) correlated with the absence of CVII in the dermal invasion associated with appendageal development. We used indirect immunofluorescent staining and affinity purified antibodies against 72-and 92 kDa type IV collagenases (gelatinases), stromelysin, matrilysin (PUMP), and interstitial collagenase. Only matrilysin was expressed by epidermal cells at the DEJ of invading appendageal cells. All other MMPs were absent in both fetal epidermis and the extracellular stroma surrounding the developing skin appendages. Further studies will be undertaken to determine whether the absence of CVII at the DEJ of appendageal buds is the result of decreased synthesis or increased degradation, perhaps by matrilysin.

SO JOURNAL OF CLINICAL INVESTIGATION, (15 NOV 1998) Vol. 102, No. 10, pp. 1788-1797.
Publisher: ROCKEFELLER UNIV PRESS, 1114 FIRST AVE, 4TH FL, NEW YORK, NY 10021.
ISSN: 0021-9738.

1/14/00
Chen, Shin-Lin
1633

L11 ANSWER 22 OF 54 SCISEARCH COPYRIGHT 2000 ISI (R)
AU TAKEUCHI T (Reprint); GASPARRO F P; BROWN D B; KONG S K; LOPRESTI N; WHITE T; CHANG P; UITTO J; BERNSTEIN E F
TI 8-METHOXYPSORALEN AND ULTRAVIOLET A RADIATION ACTIVATE THE HUMAN ***ELASTIN*** PROMOTER IN ***TRANSGENIC*** ***MICE*** - IN-VIVO AND IN-VITRO EVIDENCE FOR GENE INDUCTION
SO JOURNAL OF INVESTIGATIVE DERMATOLOGY, (APR 1996) Vol. 106, No. 4, pp. 543.
ISSN: 0022-202X.

L11 ANSWER 26 OF 54 SCISEARCH COPYRIGHT 2000 ISI (R)
AU KATCHMAN S (Reprint); WU M; COVELLO S; UITTO J
TI GLUCOCORTICOSTEROIDS UP-REGULATE HUMAN ***ELASTIN*** PROMOTER ACTIVITY IN THE SKIN OF ***TRANSGENIC*** ***MICE*** IN AN AGE-INDEPENDENT MANNER
SO JOURNAL OF INVESTIGATIVE DERMATOLOGY, (APR 1996) Vol. 106, No. 4, pp. 204.
ISSN: 0022-202X.

L11 ANSWER 29 OF 54 MEDLINE DUPLICATE 11
AU Katchman S D; Del Monaco M; Wu M; Brown D; Hsu-Wong S; Uitto J
TI A ***transgenic*** ***mouse*** model provides a novel biological assay of topical glucocorticosteroid potency.
SO ARCHIVES OF DERMATOLOGY, (1995 Nov) 131 (11) 1274-8.
Journal code: 6WU. ISSN: 0003-987X.

L11 ANSWER 31 OF 54 BIOSIS COPYRIGHT 2000 BIOSIS DUPLICATE 13
AU Del Monaco, Magaly; Wu, May; Katchman, Stacy; Tan, Elaine M. L.; Uitto, Jouni
TI Basic fibroblast growth factor regulates human ***elastin*** promoter activity in ***transgenic*** ***mice*** .
SO Journal of Investigative Dermatology, (1995) Vol. 104, No. 4, pp. 580.
Meeting Info.: Annual Meeting of the Society for Investigative Dermatology Chicago, Illinois, USA May 24-28, 1995
ISSN: 0022-202X.

MIC

L11 ANSWER 44 OF 54 MEDLINE DUPLICATE 23
AU Reitamo S; Remitz A; Tamai K; Ledo I; Uitto J
TI Interleukin 10 up-regulates ***elastin*** gene expression in vivo and in vitro at the transcriptional level.
SO BIOCHEMICAL JOURNAL, (1994 Sep 1) 302 (Pt 2) 331-3.
Journal code: 9YO. ISSN: 0264-6021.

L11 ANSWER 47 OF 54 BIOSIS COPYRIGHT 2000 BIOSIS
AU Ledo, Isabel; Mauviel, Alain; Wu, May; Hsu-Wong, Sylvia; Uitto, Jouni
TI Use of a ***transgenic*** ***mouse*** model to study the regulation of the human ***elastin*** promoter activity in vivo.
SO Journal of Investigative Dermatology, (1993) Vol. 100, No. 4, pp. 510.
Meeting Info.: Annual Meeting of the Society for Investigative Dermatology Washington, D.C., USA April 28-May 1, 1993
ISSN: 0022-202X.

Biochem. J. (1994) 302, 331–333 (Printed in Great Britain)

RESEARCH COMMUNICATION

Interleukin 10 up-regulates elastin gene expression *in vivo* and *in vitro* at the transcriptional level

Sakari REITAMO, Anita REMITZ, Katsuto TAMAI, Isabel LEDO and Jouni UITTO*

Departments of Dermatology, and Biochemistry and Molecular Biology, Jefferson Medical College, and Section of Molecular Dermatology, Jefferson Institute of Molecular Medicine, Thomas Jefferson University, Philadelphia, PA 19107, U.S.A.

In immune cells, such as T cells and monocytes, interleukin 10 (IL-10) has regulatory functions on a number of cytokines, including IL-1, IL-2, IL-8 and tumour necrosis factor-α expression. However, the effects of IL-10 have not previously been studied in detail in connective-tissue cells. In the present study, we show that recombinant human IL-10 at physiological concentrations has direct effects on the expression of the human elastin gene both *in vivo* and *in vitro*. Transgenic mice expressing a human elastin promoter/chloramphenicol acetyltransferase (CAT) reporter gene construct were injected subcutaneously with IL-10 (1–100 ng) and the site of injection was biopsied after 24 h. CAT assay revealed an increase of up to 3.5-fold in the promoter activity with 10 ng of IL-10. Transforming growth factor-β2 (TGF-β2) is known to up-regulate elastin gene expression in cultured fibroblasts. When IL-10 was added to such cultures, the effects of TGF-β2 on elastin mRNA levels were synergistically potentiated. These results suggest that IL-10 has an up-regulatory effect on elastin gene expression.

INTRODUCTION

Interleukin 10 (IL-10) was originally characterized as a factor secreted by activated mouse T helper (Th2) cell clones [1]. It was shown to inhibit synthesis of several cytokines by monocytes, and was therefore named cytokine-synthesis inhibitory factor [1]. IL-10 also suppresses the production of interferon-γ (IFN-γ) and other cytokines by Th1 cells [1,2], as well as antigen-specific proliferation of Th1 cells [3]. IL-10 is an acid-sensitive protein of 35–40 kDa [4] with structural features of a peptide segment deduced from an open reading frame in the Epstein–Barr virus genome, called BCRF1 [2,5]. In man, IL-10 is produced by activated monocytes, in addition to T cells [6]. In contrast with mouse T cells, IL-10 is produced by various human T cell subsets, including Th0, Th1 and Th2 cell clones [7,8], as well as by other cell types.

The interactions of IL-10 with other cytokines have been studied in detail in human monocytes. For example, IL-10 is a powerful suppressor of the synthesis of IL-1α, IL-1β, IL-6, IL-8 and tumour necrosis factor-α (TNF-α), as well as granulocyte–macrophage and granulocyte colony-stimulating factors in activated monocytes [6,9]. Collectively, the results of these studies indicate that the effects of IL-10 in monocytes and T cells are predominantly down-regulatory.

Essentially nothing is known about the effect of IL-10 on the expression of extracellular-matrix genes. One of the predominant components of the extracellular matrix is elastin, the major component of elastic fibres, which provides elasticity and resilience to a variety of organs, including the lungs, blood vessels and the skin [10]. Previous studies have demonstrated that transforming growth factor-β (TGF-β) up-regulates elastin gene expression *in vitro*, as determined by assay of elastin mRNA steady-state levels, whereas TNF-α and IFN-γ counteract this up-regulation [11]. It was therefore decided to examine the effects of IL-10 on elastin gene expression in combination with TGF-β and IFN-γ. For this purpose, the effects of recombinant human IL-10 on elastin gene expression were examined in dermal fibroblast cultures *in vitro*, as well as *in vivo* in transgenic mice which express a human elastin promoter/chloramphenicol acetyltransferase (CAT) reporter gene construct.

MATERIALS AND METHODS

Cell cultures

Adult human skin fibroblast cultures, established from tissue specimens obtained during cosmetic surgery, or neonatal foreskin fibroblast cultures were utilized in passages 4–10. The cultures were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal-calf serum (FCS) pretreated at 56 °C for 30 min, 2 mM glutamine, 50 μg/ml streptomycin and 50 units/ml penicillin.

Preparations of IL-10, TGF-β2 and IFN-γ

Human recombinant IL-10 was obtained from Pepro Tech Inc. (Princeton, NJ, U.S.A.) in freeze-dried form and dissolved first in distilled water, and subsequently diluted in DMEM. For cell cultures, IL-10 was used at a concentration of 10 ng/ml. For transgenic mice IL-10 was given subcutaneously in a total volume of 100 μl containing 1–100 ng of IL-10. Recombinant human TGF-β2 was kindly provided by Dr. David Olsen (Celtrix Laboratories, Santa Clara, CA, U.S.A.) and used for cell cultures in a final concentration of 10 ng/ml. IFN-γ was commercially obtained from Boehringer Mannheim (Indianapolis, IN, U.S.A.) and used for cell cultures in a final concentration of 1000 units/ml.

Transgenic mice expressing the human elastin promoter

A homozygous transgenic mouse line expressing the human elastin gene was developed by isolating a 5.2 kb fragment of the

Abbreviations used: IL-10, interleukin 10; IFN-γ, interferon-γ; TGF-β, transforming growth factor-β; TNF-α, tumour necrosis factor-α; CAT, chloramphenicol acetyltransferase; DMEM, Dulbecco's modified Eagle's medium; FCS, fetal-calf serum; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

* To whom correspondence should be addressed.

5'-flanking DNA of the human elastin gene and ligating it to a CAT reporter gene, followed by the polyadenylation signal [12]. The linearized construct was injected into fertilized oocytes of FOB/N mice, and the eggs were implanted into pseudopregnant foster mothers [13]. To test the offspring (FO) for the presence of the human elastin promoter construct, Southern blotting was used as described previously [12,14]. Mating of heterozygous animals resulted in the establishment of a homozygous mouse line. The homozygous animals express the human elastin promoter in a time-specific and developmentally regulated manner. Specifically, the highest promoter activity was noted in the lungs and aorta, tissues rich in elastin, whereas lower levels of expression were noted in the skin. Fetal and newborn animals showed higher levels of promoter activity than in adult animals [12]. Thus these mice provide a model to study the regulation of elastin gene expression at the promoter level. The animals were injected subcutaneously with IL-10 (see above), and a full-thickness 6 mm skin biopsy was obtained from the site of injection after 24 h. CAT assay of the skin biopsy was performed as described below and elsewhere [14].

CAT assays on transgenic animals

CAT activity in the skin of the transgenic animals was determined as an indicator of the expression of the elastin promoter/CAT reporter gene. The tissue specimens were homogenized in Tris/HCl, pH 7.5, with a Polytron tissue homogenizer. The homogenates were then centrifuged at 10000 *g* for 15 min at 4 °C. Protein content of the supernatants was determined with a commercial protein assay kit (Bio-Rad, Richmond, CA, U.S.A.). Portions containing 100 μg of protein were used for CAT activity determination, which was performed with [^{14}C]chloramphenicol as a substrate. The acetylated and non-acetylated forms of radioactive chloramphenicol were separated by t.l.c. [14]. The CAT activity was determined as the radioactivity in the acetylated forms as a percentage of the total radioactivity in the sample.

Northern analyses

Total RNA was isolated from confluent human fibroblast cultures incubated with or without IL-10 in medium supplemented with 1 % FCS for time periods ranging from 1 to 24 h. Total RNA (12 μg/lane) was fractionated on 0.8 % agarose gels containing 2.2 M formaldehyde, transferred to nylon filters (Zeta Probe, Bio-Rad), and immobilized by heating at 80 °C for 30 min under vacuum. The filters were prehybridized and hybridized in 50 % formamide/0.25 M NaH_2PO_4 (pH 7.2)/0.25 M NaCl/7 % (w/v) SDS/1 mM EDTA, by using cDNA probes labelled by nick translation with both [α-^{32}P]dGTP and [α-^{32}P]dCTP [15,16]. After hybridization at 42 °C for 18 h, the filters were washed in solutions with decreasing ionic strength and increasing temperature, and the final stringency of the washes was 0.1 × SSC (SSC = 0.15 M NaCl/0.015 M sodium citrate)/0.1 % SDS, at 65 °C. The cDNA–mRNA hybrids were detected by autoradiography, and the corresponding steady-state levels of mRNAs were quantified by scanning densitometry. A 3.2 kb human elastin cDNA [17] and a 1.3 kb rat glyceraldehyde-3-phosphate dehydrogenase (GAPDH) cDNA (pRGAPDH13) [18] were used sequentially for hybridizations.

RESULTS AND DISCUSSION

IL-10 up-regulates elastin gene expression *in vivo*

When transgenic mice expressing the human elastin promoter linked to CAT reporter gene were injected with different amounts of IL-10, an increase in CAT activity of up to 3.5-fold (Figure 1), and on average 3-fold (Table 1), was seen with 10 ng/ml.

Previous studies on IL-10 have mainly concentrated on the expression and effects of IL-10 in various immunocompetent cells, such as T lymphocytes and monocytes. In the present study we showed that recombinant human IL-10 is capable of up-regulating elastin gene expression *in vivo*, suggesting a possible role for IL-10 in extracellular-matrix synthesis and breakdown.

IL-10 transiently up-regulates elastin mRNA steady-state levels *in vitro*

Human skin fibroblasts in culture have been previously shown to express the elastin gene, as determined at the mRNA level. To study the effects of IL-10 on elastin gene expression, human skin fibroblast cultures maintained in 1 % FCS were incubated for up to 24 h in the presence of IL-10 at a concentration of 10 ng/ml. When different time points of incubation were studied, IL-10 showed an up-regulatory effect on elastin mRNA steady-state levels at 6 h of incubation, which was 2-fold compared with control cultures incubated without IL-10 (results not shown).

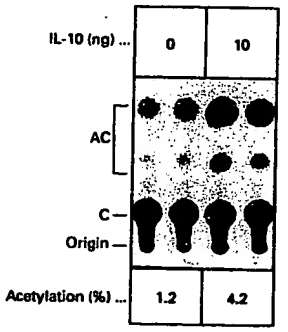


Figure 1 Enhancement of elastin promoter/CAT reporter gene activity in the skin of transgenic mice by IL-10

Transgenic mice (4 days old) were injected subcutaneously without or with 10 ng of IL-10: 24 h later, biopsy specimens of the skin were obtained from the injection site, and CAT activity was determined. An autoradiogram of the separation of the acetylated (AC) and non-acetylated (C) forms of [^{14}C]chloramphenicol by t.l.c. is shown.

Table 1 Enhancement of elastin promoter/CAT reporter gene activity by IL-10 *in vivo*

Transgenic mice (4 days old) were injected subcutaneously with IL-10: 24 h later, biopsy specimens of the skin were obtained from the injection site, and CAT activity was determined by assay of acetylated forms of [^{14}C]chloramphenicol as a percentage of total radioactivity, as shown in Figure 1.

Dose of IL-10 (ng)	No. of mice studied	CAT activity (percentage acetylation) (mean ± S.E.M.)	Relative CAT activity (% of control)
0	4	4.09 ± 0.27	100
0.1	2	4.43 ± 0.27	108
1	2	7.31 ± 4.58	178
10	4	12.07 ± 2.79	295
100	4	5.66 ± 1.02	138

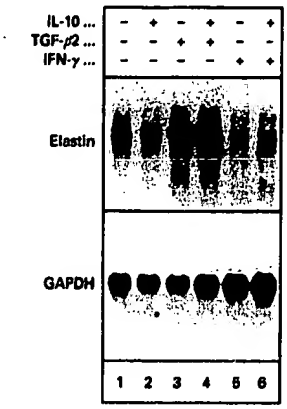


Figure 2 Effects of TGF-β2 and IFN-γ on IL-10-induced changes in elastin gene expression

TGF-β2 at 5 ng/ml and IL-10 at 10 ng/ml were added consecutively to confluent fibroblast cell strains in medium containing 1% FCS. Total RNA (12 μg/lane) was extracted after 24 h and analysed by Northern hybridizations with cDNA probes for elastin and GAPDH. Quantification of elastin mRNA levels by scanning densitometry indicated an approx. 2-fold increase by TGF-β, after correction for GAPDH mRNA levels.

TGF-β synergistically potentiates IL-10 effects *in vitro*

When dermal skin fibroblasts were incubated with IL-10 (10 ng/ml), together with TGF-β2 (10 ng/ml) or IFN-γ (1000 units/ml) for 24 h, TGF-β2 alone clearly up-regulated elastin gene expression, as shown previously [11] (Figure 2). An approximately 2-fold increase in elastin mRNA levels was seen when IL-10 was added to such cultures, based on scanning laser densitometry (Figure 2). In contrast, IL-10 had no effect on the down-regulation of elastin mRNA steady-state levels caused by IFN-γ.

At present it is not clear whether there are cells in the skin which are capable of IL-10 synthesis and secretion. As shown recently by Enk and Katz [19], murine keratinocytes secrete IL-10 when appropriately stimulated. The proximity of keratinocytes to dermal fibroblasts could result in induction of elastin by keratinocyte IL-10. Rivas and Ullrich have shown that murine keratinocytes secrete IL-10 after exposure to u.v. radiation; u.v. exposure has been shown to result in impairment of delayed-type hypersensitivity, which has been shown to be mediated, at least in part, by IL-10 [20]. Chronic exposure to u.v. also leads to changes in the composition of the matrix molecules, namely to a decrease in the amount of collagen and an increase in the amount of elastic fibres in the dermis [21,22]. The mechanisms of the u.v.-induced changes in the matrix are poorly understood. The present studies suggest that IL-10 could play a role in the pathogenesis of solar elastosis.

Both TGF-β and IL-10 have a broad spectrum of functions and have both inhibitory and stimulatory effects on diverse cells. On inflammatory murine macrophages TGF-β and IL-10 have a predominantly deactivating effect, but they suppress macrophage cytokine production under different conditions and by different mechanisms [23]. Previous studies have shown that TGF-β up-regulates elastin gene expression in pig smooth-muscle cells [24] and in human skin fibroblasts [11]. In the present study, IL-10 enhanced the up-regulatory effect of TGF-β on elastin gene expression in human dermal fibroblasts, but it did not alter the IFN-γ-elicited down-regulation of the elastin gene expression. In monocytes, IFN-γ and IL-10 antagonize each other's function [25]. The differences in the antagonistic effects of IL-10 and IFN-γ on different cell types may reflect differential signal-transduction pathways, or may be due to different *cis*-elements in the target genes.

This work was supported by U.S. Public Health Service, National Institutes of Health grants RO1-AR28450 and T32-AR07561, The Academy of Finland and Finska Läkaresällskapet. S.R. was recipient of a Fellowship from the Jefferson Center for International Dermatology at Jefferson Medical College.

REFERENCES

1 Fiorentino, D. F., Bond, M. W. and Mosmann, T. R. (1989) J. Exp. Med. **170**, 2081–2095
2 Viera, P., de Waal-Malefyt, R., Dang, M.-N., Johnson, K. E., Kastelein, R., Fiorentino, D. F., de Vries, J. E., Roncarolo, M.-G., Mosmann, T. R. and Moore, K. W. (1991) Proc. Natl. Acad. Sci. U.S.A. **88**, 1172–1176
3 de Waal-Malefyt, R., Haanen, J., Spits, H., Roncarolo, M.-G., te Velde, A., Figdor, C., Johnson, K., Kastelein, R., Yssel, H. and de Vries, J. E. (1991) J. Exp. Med. **174**, 915–924
4 Howard, M. and O'Garra, A. (1992) Immunol. Today **13**, 198–200
5 Moore, K. W., Viera, P., Fiorentino, D. F., Trounstine, M. L., Khan, T. A. and Mosmann, T. R. (1990) Science **248**, 1230–1234
6 de Waal-Malefyt, R., Abrams, J., Bennett, B., Figdor, C. G. and de Vries, J. E. (1991) J. Exp. Med. **174**, 1209–1220
7 Yssel, H., de Waal-Malefyt, R., Roncarolo, M.-G., Abrams, J. S., Lahesmaa, R., Spits, H. and de Vries, J. E. (1992) J. Immunol. **149**, 2378–2384
8 Del Prete, G., de Carli, M., Almerigogna, F., Giudizi, M. G., Biagiotti, R. and Romagnani, S. (1993) J. Immunol. **150**, 353–360
9 Fiorentino, D. F., Zlotnik, A., Vieira, P., Mosmann, T. R., Howard, M., Moore, K. W. and O'Garra, A. (1991) J. Immunol. **146**, 3444–3451
10 Rosenbloom, J., Bashir, M., Kähäri, V.-M. and Uitto, J. (1991) Crit. Rev. Eukaryotic Gene Expression **1**, 145–156
11 Kähäri, V.-M., Olsen, D. R., Rhudy, R. W., Carrillo, P., Chen, Y. Q. and Uitto, J. (1992) Lab. Invest. **66**, 580–588
12 Hsu-Wong, S., Katchman, S., Ledo, I., Wu, M., Khillan, J., Bashir, M. M., Rosenbloom, J. and Uitto, J. (1994) J. Biol. Chem. **269**, 18072–18075
13 Hogan, B., Costantini, F. and Lacy, E. (1986) Manipulating the Mouse Embryo: A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor NY
14 Sambrook, J., Fritsch, E. F. and Maniatis, T. (1989) Molecular Cloning: A Laboratory Manual, Second Edition, Cold Spring Harbor Laboratories Press, Cold Spring Harbor, NY
15 Kähari, V.-M., Fazio, M. J., Chen, Y. Q., Bashir, M. M., Rosenbloom, J. and Uitto, J. (1990) J. Biol. Chem. **265**, 9485–9490
16 Thomas, P. S. (1980) Proc. Natl. Acad. Sci. U.S.A. **77**, 5201–5205
17 Fazio, M. J., Olsen, D. R., Kauh, E. A., Baldwin, C., Indik, Z., Ornstein-Goldstein, N., Yeh, H., Rosenbloom, J. and Uitto, J. (1988) J. Invest. Dermatol. **91**, 458–464
18 Fort, P., Marty, L., Piechaczyk, M., El Sabrouty, S., Danz, C., Jeanteur, P. and Blanchard, J. M. (1985) Nucleic Acids Res. **13**, 1431–1442
19 Enk, A. H. and Katz, S. I. (1992) J. Immunol. **149**, 92–95
20 Rivas, J. M. and Ullrich, S. E. (1992) J. Immunol. **149**, 3865–3871
21 Mitchell, R. E. (1967) J. Invest. Dermatol. **48**, 203–230
22 Bernstein, E. F., Chen, Y. Q., Tamai, K., Shepley, K. J., Resnik, K. S., Zhang, H., Tuan, R., Mauviel, A. and Uitto, J. (1994) J. Invest. Dermatol. **103**, 182–186
23 Bogdan, C., Paik, J., Vodovotz, Y. and Nathan, C. (1992) J. Biol. Chem. **267**, 23301–23308
24 Liu, J. and Davidson, J. M. (1989) Biochem. Biophys. Res. Commun. **164**, 895–901
25 Chomarat, P., Rissoan, M.-C., Banchereau, J. and Miossec, P. (1993) J. Exp. Med **177**, 523–527

Received 12 May 1994/15 June 1994; accepted 22 June 1994

278879

SO JOURNAL OF CLINICAL INVESTIGATION, (15 NOV 1998) Vol 102, No. 10, pp. 1788-1797.
Publisher: ROCKEFELLER UNIV PRESS, 1114 FIRST AVE, 4TH FL, NEW YORK, NY 10021.
ISSN: 0021-9738.

1/14/00
Chen, Shin-Lin
1633

L11 ANSWER 22 OF 54 SCISEARCH COPYRIGHT 2000 ISI (R)
AU TAKEUCHI T (Reprint); GASPARRO F P; BROWN D B; KONG S K; LOPRESTI N; WHITE T; CHANG P; UITTO J; BERNSTEIN E F
TI 8-METHOXYPSORALEN AND ULTRAVIOLET A RADIATION ACTIVATE THE HUMAN ***ELASTIN*** PROMOTER IN ***TRANSGENIC*** ***MICE*** - IN-VIVO AND IN-VITRO EVIDENCE FOR GENE INDUCTION
SO JOURNAL OF INVESTIGATIVE DERMATOLOGY, (APR 1996) Vol. 106, No. 4, pp. 543.
ISSN: 0022-202X.

L11 ANSWER 26 OF 54 SCISEARCH COPYRIGHT 2000 ISI (R)
AU KATCHMAN S (Reprint); WU M; COVELLO S; UITTO J
TI GLUCOCORTICOSTEROIDS UP-REGULATE HUMAN ***ELASTIN*** PROMOTER ACTIVITY IN THE SKIN OF ***TRANSGENIC*** ***MICE*** IN AN AGE-INDEPENDENT MANNER
SO JOURNAL OF INVESTIGATIVE DERMATOLOGY, (APR 1996) Vol. 106, No. 4, pp. 204.
ISSN: 0022-202X.

L11 ANSWER 29 OF 54 MEDLINE DUPLICATE 11
AU Katchman S D; Del Monaco M; Wu M; Brown D; Hsu-Wong S; Uitto J
TI A ***transgenic*** ***mouse*** model provides a novel biological assay of topical glucocorticosteroid potency.
SO ARCHIVES OF DERMATOLOGY, (1995 Nov) 131 (11) 1274-8.
Journal code: 6WU. ISSN: 0003-987X.

MIC
Main Library
C

L11 ANSWER 31 OF 54 BIOSIS COPYRIGHT 2000 BIOSIS DUPLICATE 13
AU Del Monaco, Magaly; Wu, May; Katchman, Stacy; Tan, Elaine M. L.; Uitto, Jouni
TI Basic fibroblast growth factor regulates human ***elastin*** promoter activity in ***transgenic*** ***mice*** .
SO Journal of Investigative Dermatology, (1995) Vol. 104, No. 4, pp. 580.
Meeting Info.: Annual Meeting of the Society for Investigative Dermatology Chicago, Illinois, USA May 24-28, 1995
ISSN: 0022-202X.

L11 ANSWER 44 OF 54 MEDLINE DUPLICATE 23
AU Reitamo S; Remitz A; Tamai K; Ledo I; Uitto J
TI Interleukin 10 up-regulates ***elastin*** gene expression in vivo and in vitro at the transcriptional level.
SO BIOCHEMICAL JOURNAL, (1994 Sep 1) 302 (Pt 2) 331-3.
Journal code: 9YO. ISSN: 0264-6021.

ZGE-00011943O8

L11 ANSWER 47 OF 54 BIOSIS COPYRIGHT 2000 BIOSIS
AU Ledo, Isabel; Mauviel, Alain; Wu, May; Hsu-Wong, Sylvia; Uitto, Jouni
TI Use of a ***transgenic*** ***mouse*** model to study the regulation of the human ***elastin*** promoter activity in vivo.
SO Journal of Investigative Dermatology, (1993) Vol. 100, No. 4, pp. 510.
Meeting Info.: Annual Meeting of the Society for Investigative Dermatology Washington, D.C., USA April 28-May 1, 1993
ISSN: 0022-202X.

STUDY

A Transgenic Mouse Model Provides a Novel Biological Assay of Topical Glucocorticosteroid Potency

Stacy D. Katchman, MD; Magaly Del Monaco, DO; May Wu, MT; Douglas Brown; Sylvia Hsu-Wong, MD; Jouni Uitto, MD, PhD

Background and Design: A homozygous line of transgenic mice that expresses the human elastin promoter/CAT (chloramphenicol acetyltransferase) reporter gene construct in a tissue-specific and developmentally regulated manner is presented. Previous studies have shown that subcutaneous injections of various glucocorticosteroids up-regulate the human transgene in the mouse skin potentially through their interaction with three putative glucocorticosteroid-responsive elements contained within the human elastin promoter. In this study, we propose the use of these transgenic mice as a model system for assaying the potency of various topical glucocorticosteroid preparations.

Results: In the first set of experiments, three different commercially available topical glucocorticosteroid creams, 2.5% Hytone (2.5% hydrocortisone) (Dermik Laboratories, Fort Washington, Pa), Cutivate (0.05% fluticasone propionate) (Glaxo Inc, Research Triangle Park, NC), and Temovate (0.05% clobetasol propionate) (Glaxo Inc) (being classified into class VII, V, and I steroids, respectively) were applied to the skin of transgenic mice, with Eucerin (Beiersdorf Inc, Lindenhurst, NY) as the control cream. In a series of six experiments, Hytone 2.5% cream caused a 3.1-fold increase on the average, with Cutivate and Temovate creams resulting in 2.2-fold and 12.4-fold increases in CAT activity over control, respectively. Next, two different preparations of diflorasone diacetate 0.05% cream (Florone [class III] and Psorcon [class II], both from Dermik Laboratories), formulated with different vehicles, were compared. Psorcon caused a 22.8-fold increase in CAT activity over the control compared with a 4.4-fold increase for Florone. However, an assay comparing Psorcon ointment (class I) and Psorcon cream (class II) showed no statistically significant difference in their potencies.

Conclusions: These preliminary findings suggest the usefulness of these transgenic mice as a model system for assaying the potency of topical glucocorticosteroid preparations. Discrepancies between our data and the published classification of some topical steroids may result from anatomic differences between human and murine skin, with mouse skin being much thinner. Alternatively, the discrepancies may reflect the fact that our assay measures the biological activity of these steroids on gene transcription, while previous ranking is based on their vasoconstrictive activity.

(Arch Dermatol. 1995;131:1274-1278)

TOPICAL glucocorticosteroids are in extensive use for the treatment of a variety of inflammatory and hyperproliferative cutaneous diseases. For this purpose, a large number of glucocorticosteroids in different formulations are currently available. The effects of these steroids are based on their anti-inflammatory, immunosuppressive, antiproliferative, and vasoconstrictive activities, among other properties.[1,2] The potency of topical corticosteroids has been classified into seven (I through VII) categories, with category I being the strongest and category VII the weakest, which represent a continuum of efficacy based on their vasoconstrictive effects in a human erythema suppression test.[2-4]

Glucocorticosteroids are also potent modulators of gene expression, and, in particular, they alter the transcriptional activity of the genes expressed in cells that contain glucocorticosteroid receptors.[5] The glucocorticosteroids bind to these cytoplasmic receptors, and the receptor-ligand complexes are then translocated into the nucleus where they bind to glucocorticosteroid-responsive elements (GREs) in DNA.[5-7] As a result of this binding, the GRE-containing promoter is either up- or down-regulated. The latter interactions represent biological mechanisms that regulate various cellular functions under the influence of glucocorticosteroids.[5]

The current classification of glucocorticosteroids according to their potency is based on their vasoconstrictive activity.[3,4] In addition, a variety of other methods,

See Methods on next page

From the Departments of Dermatology and Biochemistry and Molecular Biology, Jefferson Medical College, and Section of Molecular Dermatology, Jefferson Institute of Molecular Medicine, Thomas Jefferson University, Philadelphia, Pa.

METHODS

A homozygous line of transgenic mice, which expresses 5.2 kilobases (kb) of the human elastin promoter region linked to the CAT reporter gene, was developed as described elsewhere.[8] Briefly, 5.2 kb of human elastin 5'–flanking DNA was linked to a 0.7-kb CAT gene, followed by 0.3 kb of DNA with a polyadenylation signal. This linearized construct was injected into fertilized oocytes, and a line of transgenic mice expressing the human elastin promoter, as detected by CAT activity, was developed. These transgenic mice have no clinical phenotype, and they do not express human elastin protein, as no part of the coding sequence is contained within the transgene. Our previous experiments[8] have shown that the human elastin promoter/CAT construct is expressed in a tissue-specific and developmentally regulated manner.

We used 4- or 5-day-old hairless pups, homozygous for the transgene, as test animals. In each experiment, pups from the same litter were used for comparison between glucocorticosteroid and control preparations in parallel. The commercial glucocorticosteroid preparations were tested by applying 0.03 g uniformly on their dorsal surface (≈14 mg/cm^2). In some experiments, the steroid preparation was mixed in varying proportions with Eucerin (Beiersdorf Inc, Lindenhurst, NY) as an inert vehicle; Eucerin alone did not alter the CAT activity in the skin. Either a single application or two applications, one at time point 0 and another one at 12 hours, were used. Control animals received the same amount of Eucerin cream and were examined in parallel. The test animals were separated from each other and from their mothers, were killed at different time points, and skin biopsy specimens were removed immediately from the treated area. For the CAT assay, skin was homogenized with a polytron tissue homogenizer in 0.25-mol/L TRIS hydrochloride at a pH of 7.5. The homogenates were then freeze thawed three times and centrifuged at 10 000g for 15 minutes. The protein content of the supernatants was determined by a commercial kit (Bio-Rad Laboratories, Hercules, Calif), and aliquots, containing 100 μg of protein, were assayed for CAT activity in the linear range of the assay,[11,12] reflecting the human elastin promoter activity.[8] The significance of differences between different treatment groups was evaluated by Student's *t* test, using a computer program (Statworks, Cricket Software Inc, Philadelphia, Pa).

based on their anti-inflammatory and antiproliferative activities, have been proposed.[1] In this article, we describe a novel in vivo model to test the potency of topical glucocorticosteroids based on their biological activity. This model consists of a transgenic mouse line that expresses a human elastin promoter/CAT (chloramphenicol acetyltransferase) reporter gene construct in a tissue-specific and developmentally regulated manner.[8] The basis for this assay is the discovery that the human elastin promoter responds in a dose- and time-dependent manner to subcutaneous injections of triamcinolone acetonide and dexamethasone.[9] This response is probably mediated by the three putative GREs present in the promoter region.[10] Since CAT is a bacterial enzyme not present in vertebrate tissues, the assay of CAT activity in the transgenic animals directly reflects the activity of the human elastin promoter.[8] In this preliminary study, we have tested several topical glucocorticosteroids to validate this assay system.

RESULTS

The transgenic mouse model used in this study has previously been shown to express the human elastin promoter/CAT reporter gene construct in a tissue-specific and developmentally regulated manner.[8] Specifically, relatively high levels of activity were noted in the lungs and the aorta, tissues rich in elastin, while lower levels were noted in the kidneys and the skin. Recent studies[9] have also shown that subcutaneous injection of triamcinolone acetonide or dexamethasone can markedly increase the elastin promoter activity in the skin of these animals in a dose- and time-dependent manner. In this study, we tested topically applied glucocorticosteroids for their effects on the elastin promoter activity in the skin, as determined by CAT assay.

To validate the reproducibility of the assay, the intrasubject variability was first assessed by assaying the same skin extract sample in seven parallel determinations. The variability of the assay was ±12.5% SDs from the mean. Second, the CAT values reflecting intersubject variability within mice representing the same litter were found to be 2.83%±0.89% (mean±SD) in three separate experiments, each performed in triplicate.

Optimal time for skin biopsies was determined by measuring the CAT activity at different time points after a single application of Temovate (0.05% clobetasol propionate) (Glaxo Inc, Research Triangle Park, NC), a potent enhancer of the CAT activity (see below). Only a modest, approximately 1.6-fold, enhancement was noted at 6 hours, while the increases in CAT activity at 12-, 24-, and 48-hour points were 5.1-, 23.2-, and 49.8-fold, respectively, over the 0-hour point (each value being the mean of two parallel determinations in individual animals). Thus, taking into consideration the limited viability of the pups beyond 48 hours, when separated from their mothers, the subsequent assays were performed at the 24-hour point.

In the first set of comparative experiments, we tested three different, commercially available topical glucocorticosteroid creams, ie, Hytone (2.5% hydrocortisone) (Dermik Laboratories, Fort Washington, Pa), Cutivate (0.05% fluticasone propionate) (Glaxo Inc), and Temovate (0.05% clobetasol propionate). These three glucocorticosteroid preparations have been classified according to their vasoconstrictive activity into classes VII, V, and I, respectively.[4] In control animals, which received Eucerin cream alone, CAT assay demonstrated a low, yet clearly detectable, level of activity (**Figure 1**). However, the CAT activity in the Eucerin-treated animals was not different from that measured in untreated skin. Similarly, the topical application of petrolatum did not alter the expression of CAT activity in the skin (not shown). As illustrated by Figure 1, application of any of the three glucocorticosteroids clearly enhanced CAT activity in the treated area of the skin. However, there was a difference in the response. In six inde-

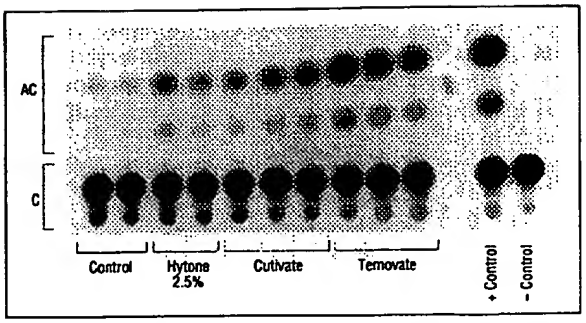


Figure 1. *Determination of potency of three commercially available topical glucocorticosteroid preparations: Hytone 2.5% (Dermik Laboratories, Fort Washington, Pa) and Cutivate and Temovate (both from Glaxo Inc, Research Triangle Park, NC). Five-day-old transgenic mice expressing the human elastin promoter/CAT (chloramphenicol acetyltransferase) reporter gene construct were treated with 0.03 g of the indicated commercial topical glucocorticosteroid applied to their dorsal skin, with Eucerin cream (Beiersdorf Inc, Lindenhurst, NY) as the control, at 0 and 12 hours. At 24 hours, the mice were killed and skin biopsy specimens were immediately obtained from the treated areas and CAT activity was determined using 100 μg of protein extracted from the skin biopsy specimens in a 16-hour incubation with chloramphenicol tagged with carbon 14 (^{14}C), as described elsewhere.[9] The figure is an autoradiogram illustrating the separation of acetylated (AC) and nonacetylated (C) forms of radioactive chloramphenicol by thin-layer chromatography. The control samples included an extract of lung from a homozygous untreated animal as a positive control (plus sign), and the negative control (minus sign) consisted of tissue extract from a wild-type mouse. The CAT activity was quantitated by counting the radioactivity in AC and C forms of [^{14}C]chloramphenicol and expressed as the percentage of acetylation. The relative CAT activities in animals receiving the different steroid preparations were calculated in relationship to control animals. Each lane represents an assay from a separate animal within a litter of homozygous transgenic animals.*

pendent trials, each using mice representing a single litter, Hytone 2.5% increased CAT activity 3.1±0.9-fold over the control (mean±SD from six different experiments). In the same six experiments, Cutivate showed a 2.2±0.6-fold increase, while Temovate resulted in a 12.4±3.2-fold increase on the average. It should be noted that within each experiment, the compounds were tested in up to five individual animals, each assayed separately in parallel.

To examine the effects of different formulations of the glucocorticosteroids on CAT activity, two types of additional tests were performed. First, diflorasone diacetate, formulated in 0.05% concentration in two different commercial cream preparations (Florone and Psorcon [both from Dermik Laboratories]), which have been categorized in classes III and II, respectively, was tested. For comparison, 1% hydrocortisone (Hytone 1%, Dermik Laboratories), a class VII steroid, was used in parallel on three animals. Application of Hytone 1% resulted in a 5.9±0.8-fold (mean±SD; n=3) increase in CAT activity (**Figure 2**). Parallel animals treated with Psorcon depicted a 22.8±3.1-fold increase in CAT activity. However, the same glucocorticosteroid in a different formulation (Florone) resulted only in a 4.4±0.6-fold increase in CAT activity (Figure 2). Enhancement of CAT activity by all three steroids was statistically different from that in controls ($P<.001$). However, the activity in the animals treated with Hytone 1% and Florone did not significantly differ from each other ($P=.063$), while Psorcon was clearly superior over the other two steroid preparations tested ($P<.001$). Thus, the formulation of the glucocorticosteroids used for the assay apparently has a significant effect in this system.

In the second set of experiments, examining the effects of different formulations, diflorasone diacetate (Psorcon) was tested in cream and ointment vehicles corresponding to classes II and I, respectively. In two separate experiments, no significant difference in the CAT activity between the animals treated either with the cream or the ointment form of Psorcon was noted. Specifically, the average ratio of enhancement of CAT activity with cream vs ointment preparations in two separate experiments, each performed in triplicate, was 1.19 ($P>.05$).

Since the amount of the glucocorticosteroid preparations used for topical application was relatively high (14 mg/cm^2), the most potent steroids could conceivably saturate the receptor-mediated signaling pathway. To exclude this possibility, Temovate, the most potent enhancer of CAT activity tested (see above), was mixed with Eucerin in varying proportions (1:3, 1:9, or 1:18 by weight). By increasing the dilution of Temovate by Eucerin, a proportional decrease in the enhancement of CAT activity was noted. Specifically, the enhancement of CAT activity at 1:3, 1:9, and 1:18 dilutions was 3.1-, 1.4-, and 1.0-fold compared with that in the Eucerin-treated control animals.

COMMENT

Topical corticosteroids are among the therapies most widely used by dermatologists today.[2] There is a multitude of different potencies and formulations that are used clinically for a variety of inflammatory and hyperproliferative diseases. Consistent with the broad range of clinical applications for topical corticosteroids is also the broad range of actions by which they exert their biological effects. Some of the proposed mechanisms of action for the anti-inflammatory effects of these products include promotion of vasoconstriction, inhibition of phagocytosis, stabilization of lysosomal membranes of phagocytizing cells, and interruption of the prostaglandin cascade by preventing the release of arachidonic acid.[1] Additionally, topical corticosteroids are also useful for their antiproliferative effects that are mediated by inhibition of DNA synthesis and mitosis. This results clinically in thinning of the epidermis, which is often a desirable end point in the treatment of certain hyperproliferative disorders, such as psoriasis. On the other hand, glucocorticosteroids prevent the synthesis of collagen by fibroblasts, resulting in atrophy of the dermis, an untoward side effect of long-term glucocorticosteroid application.[13,14]

Many different models have been developed for the assay of topical corticosteroid potency. Each model is able to highlight only one particular effect from the broad spectrum of glucocorticoid action.[1] For example, the test that is most commonly used today to assay the potency of topical steroid preparations is the vasoconstrictor assay of McKenzie and Stoughton[3] and Stoughton[4] This assay assesses the ability of alcoholic solutions of a glucocorticosteroid applied to intact human skin to produce vasoconstriction, as seen by blanching of the skin. Other tests of corticoid potency rely on the ability to inhibit blister and pustule formation,[14] the ability to prevent a cell-mediated immune response to challenge with *Rhus* antigen,[15] the ability to heal psoriasis plaques,[16] and the ability to produce skin atrophy and telangiectasia.[17]

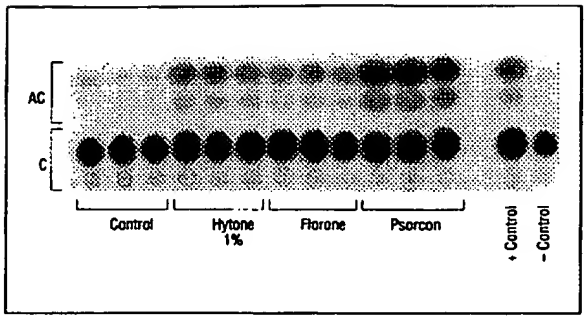


Figure 2. *Up-regulation of human elastin promoter activity in the skin of transgenic mice treated with Hytone 1%, Florone, and Psorcon creams (all from Dermik Laboratories, Fort Washington, Pa). The mice were treated with topical steroids, and CAT (chloramphenicol acetyltransferase) activity was determined at 24 hours, as described in Figure 1. Each lane indicates an assay from a separate animal within a litter of homozygous transgenic animals; AC and C, acetylated and nonacetylated, respectively, forms of chloramphenicol; positive (plus sign) and negative (minus sign) controls are included, as in Figure 1.*

In this study, we propose a novel biological assay for determining the potency of topical corticosteroid preparations using transgenic mice that have incorporated a human elastin promoter–CAT reporter gene construct into their genome. Thus, we can take advantage of the three putative GREs contained within the elastin promoter,[10] as they represent elements that respond to glucocorticosteroids in a dose-dependent manner, and this response can then be quantitated by CAT assay. This model's main advantage is that it can directly assay the biological action of the glucocorticosteroids in the skin. Other previously published methods rely on indirect or noncomprehensive means to determine corticosteroid potency, such as determination of vasoconstriction or prevention of blister formation.

The goals of our study were threefold. First, we performed an experiment to validate the assay and assess its reproducibility. Second, we wanted to compare, in a preliminary manner, the potency of selected commercially available topical steroid preparations in our assay system with their established potencies, as based on their vasoconstrictive ability. Third, we wanted to assess the potential importance of the glucocorticosteroid vehicle in contributing to the potency of a particular preparation.

To validate the transgenic mouse model assay, we first determined both intrasubject and intersubject variability within a litter of the CAT assay. The results indicated good reproducibility (SD being 31.4% of the mean; n=9) in representing the same litter, a finding that clearly allowed detection of statistically significant differences between control and glucocorticosteroid-treated animal groups. It should be noted that each experiment uses control and treated mice from a single litter, all assays being performed in parallel, thus allowing direct comparisons between the treatment groups. It should also be noted that the test animals represent a homozygous transgenic mouse line, the copy number of the transgene (seven per mouse genome) being stable.[9] We further demonstrated that even with the most potent enhancer of CAT activity tested (Temovate cream, class I preparation), the assay system was not saturated, since dilution of this steroid preparation by an inert cream (Eucerin) porportionally reduced the CAT activity in the skin. On the other hand, use of a relatively weak, class VII, glucocorticosteroid preparation (Hytone 1%) clearly enhanced the CAT activity compared with that in the control animals, but the enhancement was significantly less than that noted with Temovate. Thus, the transgenic mouse model presented in this study is able to discriminate these two glucocorticosteroid preparations from controls and from each other.

To demonstrate the feasibility of this assay system, selected commercially available glucocorticosteroid preparations were tested. Using Hytone 2.5%, Cutivate, and Temovate creams, with Eucerin cream as the control, our model system revealed Hytone 2.5% and Temovate to be 3.1 and 12.2 times, respectively, more potent than the control cream. This result is consistent with the published potencies of these preparations, those being classes VII and I for Hytone 2.5% and Temovate creams, respectively. However, our results indicated that Cutivate potency was only 2.2 times higher than that of the control, a finding that is not consistent with its established rank as a class V topical steroid. Furthermore, in a parallel experiment comparing Hytone 1%, Florone, and Psorcon creams, Hytone 1% and Psorcon exhibited CAT activities of 5.9 and 22.8 times that of the controls, respectively, consistent with their known relative strengths as class VII and class II steroids. However, the medium-strength preparation, Florone cream, which is a class III steroid, exhibited a CAT activity of only 4.4 times that of the control.

The third part of our study, ie, the assessment of the importance of vehicle, yielded interesting results. For example, Psorcon cream and ointment, classes II and I, respectively, did not show statistically significant differences in the CAT activities, and thus their potencies were equivalent by our assay system. However, Florone and Psorcon creams, which both contain the same active ingredient (0.05% diflorasone acetate), but are formulated in different bases, displayed markedly different CAT activities. In this case, Florone cream exhibited much weaker CAT activity than did Psorcon cream. These results emphasize that the vehicle of a glucocorticosteroid may have a major influence on the biological activity and tissue availability of the steroid preparation.

In summary, we have devised a novel in vivo technique for the assay of topical steroid potencies. The major limitations of our model include the significant histologic and pharmacokinetic differences between mouse and human skin. For example, murine epidermis is much thinner than human epidermis and may be more easily penetrated by glucocorticosteroids. This limitation is highlighted by the fact that we used in our assay newborn mice (4 or 5 days postnatal), which may not have acquired full epidermal barrier function. Thus, the findings of this study should be considered preliminary with respect to establishing this approach as a valid method for the assay of relative steroid potency for human skin. Nevertheless, the use of newborn mice in this assay system is necessitated by the fact that these animals grow hair beginning about the sixth day of their life, and the presence of hair in older animals interferes with accurate application of the steroid preparations and compromises the accuracy of protein determinations for CAT assay. Further limitations of this method include the relatively small litter size (usually less than 12 pups) that limits the number of different compounds that can be tested in a single assay. Finally, since the newborn

animals, when separated from their mothers, have a limited viability, the length of the treatment time is reduced to less than 48 hours. Thus, this assay is not suitable for long-term testing of pharmaceutical compounds.

The advantages of our test system over previously used methods include its ability to make quantitative, rather than qualitative, comparisons, such as those in the vasoconstrictor assay that are based on subjective evaluation of the redness in individuals by an observer,[3,4] although the latter assay has been suggested to correlate with clinical efficiency of topical steroids.[18] Furthermore, our assay system tests for the biological action of the glucocorticosteroid on a GRE, an element specifically responsive to steroids, in a dose-dependent manner, rather than relying on techniques measuring indirect effects, such as the prevention of blisters. The transgenic mouse system is also quick and can yield quantitative data within 72 hours. This feature makes this assay system particularly suitable for the rapid screening and development of pharmaceutical compounds for their potential usefulness as topical glucocorticosteroid preparations. Finally, this mouse model represents a prototypic biological assay system for glucocorticosteroids, and similar approaches can be taken to develop mice with transgenes that contain response elements to other classes of compounds, such as retinoids and vitamin D derivatives.

Accepted for publication April 6, 1995.

This study was supported by the US Public Health Service, National Institutes of Health grants AR-28450 and T32-AR0751, and by the Dermatology Foundation, Evanston, Ill.

Joel Rosenbloom, MD, PhD; Muhammad Bashir, PhD; Jaspal Khillan, PhD; and Machiko Arita, MS, participated in the development of the transgenic mice. We thank Tamara Alexander for secretarial assistance.

Reprint requests to Department of Dermatology, Jefferson Medical College, 233 S 10th St, Room 450 BLSB, Philadelphia, PA 19107-5541 (Dr Uitto).

REFERENCES

1. Wendt H, Frosch PJ. *Clinico-Pharmacological Models for the Assay of Topical Corticoids: Comparative Trials With Diflucortolone Valerate.* Basel, Switzerland: S Karger AG; 1982:3-61.
2. Maibach HI, Surber C, eds. *Topical Corticosteroids.* Basel, Switzerland: S Karger AG; 1992:17-53, 154-161.
3. McKenzie AW, Stoughton RB. Method for comparing percutaneous absorption of steroids. *Arch Dermatol.* 1962;86:608-610.
4. Stoughton RB. Vasoconstriction assay. In: Maibach M, Surber C, eds. *Topical Corticosteroids.* Basel, Switzerland: S Karger AG; 1992:42-53.
5. Beato M. Gene regulation by steroid hormones. *Cell.* 1989;56:335-344.
6. Su Y, Pitot HC. Location and characterization of multiple glucocorticoid-responsive elements in the rat serine dehydratase gene. *Arch Biochem Biophys.* 1992;297:239-243.
7. Petersen DD, Magnuson MA, Granner DK. Location and characterization of two widely separated glucocorticoid response elements in the phosphoenolpyruvate carboxykinase gene. *Mol Cell Biol.* 1988;8:96-104.
8. Hsu-Wong S, Katchman SD, Ledo I, et al. Tissue-specific and developmentally regulated expression of human elastin promoter activity in transgenic mice. *J Biol Chem.* 1994;269:18072-18075.
9. Ledo I, Wu M, Katchman S, et al. Glucocorticosteroids upregulate human elastin gene promoter activity in transgenic mice. *J Invest Dermatol.* 1994;103:632-636.
10. Kähäri VM, Fazio MJ, Chen YQ, Bashir MM, Rosenbloom J, Uitto J. Deletion analyses of 5'-flanking region of the human elastin gene: delineation of functional promoter and regulatory *cis*-elements. *J Biol Chem.* 1990;265:9485-9490.
11. Gorman CM, Moffat LF, Howard BH. Recombinant genes which express chloramphenicol acetyltransferase in mammalian cells. *Molec Cell Biol.* 1982;2:1044-1051.
12. Sambrook J, Fritsch EF, Maniatis T. *Molecular Cloning: A Laboratory Manual.* 2nd ed. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory; 1989.
13. Oikarinen AI, Uitto J. Molecular mechanisms of glucocorticoid action on connective tissue metabolism. In: Uitto J, Perejda A, eds. *Connective Tissue Disease: Molecular Pathology of the Extracellular Matrix.* New York, NY: Marcel Dekker; 1987:385-397.
14. Kaidbey KH, Kligman AM. Assay of topical corticosteroids by suppression of experimental inflammation in humans. *J Invest Dermatol.* 1974;63:292-297.
15. Kaidbey KH, Kligman AM. Assay of topical corticosteroids: efficacy of suppression of experimental *Rhus* dermatitis in humans. *Arch Dermatol.* 1976;112:808-810.
16. Dumas KJ, Scholtz JR. The psoriasis bio-assay for topical corticosteroid activity. *Acta Dermatovenereol (Stockh).* 1972;52:43-48.
17. Frosch PJ, Kligman AM, Wendt H. Tests for the assay of skin atrophy: Dühring Chamber test for telangiectasia. In: Mauvais-Jarvis P, Vickers CRH, Wepierre J, eds. *Percutaneous Absorption of Steroids.* Orlando, Fla: Academic Press Inc; 1980:185-198.
18. Cornell RC, Stoughton RB. Correlation of the vasoconstriction assay and clinical activity in psoriasis. *Arch Dermatol.* 1985;121:63-67.